JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXXI WASHINGTON, D. C., SEPTEMBER 15, 1925 No. 6

# PLATE COUNTS OF SOIL MICROORGANISMS [1]

By N. R. SMITH, *Associate Bacteriologist*, and S. WORDEN, *formerly Junior Scientific Aid, Bureau of Plant Industry, United States Department of Agriculture* [2]

## INTRODUCTION

Although plate counts of the microorganisms in soil samples have only restricted value, nevertheless, improvements in the method for making such determinations are desirable. Recently recommendations have been made on three points, (1) procedure in preparing the suspension of the soil organisms, (2) the use of special media for plating, and (3) frequent repetitions of the platings. These recommendations have been tested experimentally, and the results are presented in this paper.

## METHODS FOR PREPARING SUSPENSIONS OF SOIL MICROORGANISMS

The intensity with which soil samples suspended in water should be shaken has been tested repeatedly, and it was the general belief that thorough shaking by hand for about five minutes gives a satisfactory suspension. The efficiency of this method, however, has been questioned by Whittles (*14*),[3] who subjected soil suspended in water to a high frequency vibration with an electric vibrator. On plating out from these vibrated suspensions, he obtained counts widely surpassing those obtained with the hand-shaking method. In his first experiments with a soil which gave about 10 million organisms per gram by the usual plating procedure, he counted 250 millions per gram soil from the vibrated suspension. Later platings with a modified technique gave even counts of 100 and 270 colonies per plate from the $1-10^{11}$ dilution equal to 10 and 27 billion [4] organisms per gram soil.

The colonies in this case attained their maximum growth within 4 days, and there were no "slow growers." Whittles suggested that this was due to the freeing of the bacteria from the enveloping colloidal gel which contained their metabolic products. The dispersion of the gel by the vibration was declared to have removed the inhibiting influence and to have caused the rapid growth. An "alkaline shake medium" containing a protective colloid (gelatin) was found to be helpful in bringing about a good dispersion. For comparison, direct microscopic counts were made in the soil samples. The numbers recorded (6,300 millions per gram) are much higher than those usually found in soil tests and agree well with the lower vibrator counts.

[1] Received for publication September 18, 1924; issued October, 1925.
[2] The writers are especially indebted to F. Löhnis for many helpful suggestions and for critical reading of the manuscript, and to F. L. Goll for the drawing of the curves.
[3] Reference is made by number (italic) to "Literature cited," pp. 516.
[4] In England and Germany the term billion is used for a million millions and not for a thousand millions as in America. Billion in this paper is a million millions.

It was obvious from the start that the high numbers recorded could not be correct. The volume of 1 gm. of soil is ordinarily a little less than 1 c. c. This space would be completely filled by approximately 1,000,000,000,000 (1 billion) bacteria (*6 p. 17*). The 10 or more billions [4] per gram counted by Whittles undoubtedly originated from outside contaminations (*11*). But there still remained the possibility that after eliminating this source of error the use of the vibrator might insure a more perfect distribution of the soil organisms than is obtainable by shaking the soil dilutions by hand.

### THE VIBRATOR APPARATUS

Whittles' vibrator apparatus consists mainly of four parts: A vibrator cup, an electric vibrator, a reservoir for sterile diluent, and a sterile receiving flask. The writers constructed a similar apparatus in the following manner.

The vibrator cup was made from a large-mouthed glass jar 2¼ inches in diameter, having an aluminum screw cap. The bottom of the jar was cut off, and the center of the cap removed, leaving only enough of it to make a good seal when screwed on. A disk of pyralin was fitted into this cap and a soft rubber gasket placed on each side to prevent leaking. When the jar is inverted the disk of pyralin becomes the bottom of the vibrator cup and the vibrating membrane. For the outlet, a hole was drilled in the side of the cup at the 100 c. c. level and a right-angle tube with a rubber gasket inserted.

The vibrator was simply an ordinary electric door bell with the bell removed. A rubber stopper was placed under the hammer so that by raising or lowering it, adjusting the screw of the make-and-break and the height of the vibrator cup above the hammer, the correct vibration of high frequency could be produced, violently agitating the surface of the liquid in the cup.

The reservoir was a 2-liter Erlenmeyer flask having a siphon long enough to reach nearly to the bottom of the vibrator cup. The writers found it most convenient to sterilize the filled reservoir for 1 hour in the autoclave at 20 pounds pressure. In order to be able to do this, the lower end of the siphon had to be flexible, so that it could be turned upward on itself, closed with a sealed rubber tubing, and wrapped in cotton. When ready for use, a screw clamp on the flexible portion controlled the flow through the siphon. The receiving flask was a sterile 2-liter flask graduated at 1,900 c. c.

### STERILIZATION OF THE VIBRATOR

The sterilization of the vibrator cup, with its pyralin membrane and rubber gaskets, could not be carried out in the autoclave or hot-air oven, nor could salts of the heavy metals be used. Alcohol seemed to fulfill the requirements and was tried in the first experiments. The vibrator cup was taken apart, soaked for 20 minutes in 50 per cent ethyl alcohol, rinsed with sterile water, and assembled under aseptic conditions. It was then placed in position over the vibrator, fastened with a clamp, filled with sterile water, and the vibrator started. As a check on the sterility of the cup, four 1 c. c.

[4] See footnote on p. 1.

portions were withdrawn and plated. One gram of a clay loam field soil was put into the cup, and after a minute or two of vibration the siphon from the reservoir was started. The flow was adjusted so that the 1,900 c. c. would pass through in from 20 to 30 minutes. At the end, the suspension was mixed and further dilutions were made in sterile tap water in steps of 10×, by putting 5 c. c. in 45 c. c. Four plates from the 1:200,000 dilution were poured on soil extract agar.[5] As a check on the effect of the vibrator, 10 gm. of the soil were added to 1,000 c. c. sterile tap water and shaken vigorously from 3 to 5 minutes. Dilutions were made in steps of 10× by adding 5 c. c. to 45 c. c., reaching the final dilution of 1:100,000 from which four plates were poured. (Data in Table I.)

TABLE I.—*Results of the first plating, together with series 2 which was made as a control to series 1*

| Series | Suspension by— | Dilution | Millions per gram soil | Control from cup, per c. c. |
|---|---|---|---|---|
| 1 | Hand | 1:100,000 | 7.5 | ---------- |
| | Vibrator | 1:200,000 | 14.6 | *5,000 |
| 2 | Hand | 1:100,000 | 13.1 | ---------- |
| | Vibrator | 1:200,000 | 22.6 | 4,200 |
| | Vibrator | 1:200,000 | 15.4 | 6,500 |

* Estimated.

A glance at the last column of Table I is enough to condemn alcohol as the sterilizing agent of the vibrator cup. Although the effect of this contamination on the final dilution is small, it is a fruitful source of error.

The next method of sterilizing consisted in soaking the parts of the vibrator cup in a solution of equal parts of formaldehyde and water for 15 minutes. The parts were then rinsed with sterile water, assembled aseptically, put in place over the vibrator, and filled with sterile water. At this point the gallic acid test for formaldehyde was made with 2 c. c. water from the cup.[6] If the test was positive, sterile water was run through until it became negative. One gram of soil was then added, and vibrated as in the preliminary experiment.

The first trials with formaldehyde showed that the apparatus could be rendered practically sterile. It was the writers' custom, however, to make four plates from the vibrator cup before every set of experiments, thus positively checking on the sterilization. In 13 different tests, representing a total of 52 plates, an average of only 2 colonies per plate was found, the highest number recorded being 7, while many plates were sterile.

Whittles (*14*) used bleaching powder for the sterilization of the vibrator, and the writers tested this substance, too. Following his directions, the cup was taken apart, "thoroughly washed in running water under the tap, rinsed with a solution of bleaching powder to which a drop of hydrochloric acid had been added; it was again washed thoroughly in running water, and then with a dilute solution

[5] Field soil 500 gms., water 1,200 c. c., autoclave half hour at 15 pounds, or boil over a bare flame for 15 minutes. Filter, make up to 1,000 c. c., add 0.5 gms. $K_2HPO_4$ and 20 gms. agar. Adjust to $P_H$ 7 to 7.2.

[6] To 2 c. c. sample add a few drops of a saturated alcoholic (absolute) solution of gallic acid and stratify over concentrated sulphuric acid. A blue ring indicates formaldehyde. Sensitive to 1 part in 200,000.

of sodium thiosulphate and again with distilled water; finally with some sterile shake medium." [7] Two soils were used for these tests. One gram in each case was vibrated with 2,000 c. c. diluent, then 2 c. c. of this suspension again vibrated with 2,000 c. c., making a final dilution of 1 : 2,000,000. The hand-shaking control plates of these soils gave 12 and 10 millions per gram soil, respectively. The analogous data for the vibrated suspensions were as follows: First test, 554 millions and 26 millions; second test, 47.5 millions and 41.2 millions.

Although these counts are much lower than those made by Whittles, they suffice to show that undoubtedly the error lay, in his case, in the incomplete sterilization of the vibration cup.

### EFFICIENCY OF VIBRATION

After having ascertained that the use of formaldehyde assures a satisfactory sterilization of the vibrator, the writers' attention was turned to the effect of the vibration and to the value of the "alkaline shake medium" recommended for making soil suspensions. The shake medium was prepared by diluting 50 c. c. of the stock solution (Na Cl 10 per cent : $Na_2CO_3$ 2.5 per cent) to 4 liters, which were sterilized. As inoculum two clay soils were chosen (Nos. 109 and 260). These had been in the laboratory for more than a week. Methods of making dilutions and plating were the same as in the preliminary experiments. The results of the nine tests are presented in Table II.

TABLE II.—*Influence of vibration and "shake media" on total counts*

| Suspension by— | Dilution | Sterile diluent | Soil 109 millions per gram | Soil 260 millions per gram |
|---|---|---|---|---|
| Hand shaking | 1 : 250,000 | Tap water | 16.7 | 13.0 |
| Vibrator | 1 : 200,000 | do | 18.8 | 12.6 |
| Do | 1 : 200,000 | Whittles' shake medium | 16.6 | 11.8 |
| Do | [a]1 : 2,000,000 | do | [a] 13.0 | [a] 20.0 |
| Do | [a]1 : 2,000,000 | Whittles' medium, plus 0.1 per cent gelatin | [a] 18.0 | [a] 18.0 |
| Do | 1 : 200,000 | Whittles' shake medium, plus 0.1 per cent agar | 15.9 | 11.3 |
| Do | 1 : 200,000 | Whittles' shake medium, plus 0.1 per cent gelatin | 11.1 | 10.6 |
| Hand shaking | 1 : 250,000 | Tap water | 11.6 | 10.1 |

[a] Dilutions were made by placing 2 c. c. of the first dilutions back into the vibrator cup and repeating the vibration. The colonies were not numerous enough for accurate counting.

These analyses extended over a period of several days and were made in the order given in the table. That there was very little change in the total number of microorganisms is shown by the close agreement of the hand-shaking control made at the beginning and at the end of the series. If the counts from the 1 : 2,000,000 dilutions, which were too high, are excluded, the same agreement is evident throughout the table, whether a "shake medium" or tap water was used as diluent, and whether the suspension was made by the hand-shaking or by the vibrator method. Furthermore, the addition of a protective colloid to the suspension, such as 0.1 per cent gelatin or agar, gave no higher counts than when sterile tap water was used as diluent.

[7] Nothing is said in these directions about the necessity of using not simply "distilled water," but "sterilized distilled water."

All the counts given in Table II were made on plates poured with soil extract agar. As Whittles used a "mannite-salts agar" (*14, p. 19*), it was necessary to adopt this medium to get fully comparable results. At the same time, it seemed desirable to check up the results given in Table II as to the efficiency of the vibration compared with hand shaking and also in regard to the influence of the diluent. The inoculum in this case was a clay-loam field soil.

TABLE III.—*Comparison of mannite-salts and soil-extract agars, tap water and shake medium, vibration and hand shaking*

| Suspension by— | Dilution | Diluent | Mannite salts agar | Soil extract agar | Average | Average |
|---|---|---|---|---|---|---|
| | | | *Millions per gm.* | *Millions per gm.* | | |
| Hand shaking | 1 : 250,000 | Tap water | 8.9 | 10.8 | 9.9 | 9.7 |
| Do | 1 : 250,000 | Shake medium | 8.3 | 10.6 | 9.5 | |
| Vibrator | 1 : 200,000 | Tap water | 11.5 | 16.6 | 14.0 | 11.9 |
| Do | 1 : 200,000 | Shake medium | 7.7 | 11.8 | 9.8 | |
| Average | | | 9.0 | 12.4 | | |

A comparison of the counts made on mannite-salts agar and on soil-extract agar, as recorded in Table III, shows a small but distinct difference in every case in favor of soil-extract agar. With the soil-extract agar, the colonies showed less tendency to spread, and on the whole they were easier to count. Tap water used as diluent gave consistently higher counts, although the difference is small in the hand-shaking series. When vibrator and hand shaking are compared, the former gave a somewhat higher average, but the counts making up this average are much more erratic than those in the hand-shaking series, and consequently less valuable.

In this connection, the fact should not be overlooked that growth might take place in the vibrator or in the suspension. When making the experiments, not less than 45 minutes elapsed between the addition of the soil to the vibrator cup and the plating out of the highest dilution. In order to ascertain whether growth actually amounts to much under these conditions, the following test was made. A three-day-old agar slant culture of *Bacterium fluorescens* was flooded with sterile water, the growth scraped off, and this suspension transferred to a flask of sterile water. After shaking for three minutes, four aliquots were taken out, two of which were diluted by hand shaking, one in tap water and the other in shake medium with gelatin; and two were vibrated with the same diluents. Plates were poured from the 2 and 2,000,000 dilutions, incubated at 28° C. and counted after three days with the following results:

1. Control, diluted with tap water, 600 millions.
2. Control, diluted with shake medium (gelatin), 540 millions.
3. Vibrated, diluted with tap water, 135 millions.
4. Vibrated, diluted with shake medium (gelatin), 690 millions.

The vibrator cup leaked at the beginning of the first run and some of the inoculum was lost; therefore, the count of 135 millions can be disregarded. In spite of this accident, it can safely be assumed that growth is not responsible for a marked increase, especially if only one suspension is made in the vibrator, as in the case just given.

In making the suspensions, the even dispersion of the soil particles by the vibrator was very evident. The suspensions did not contain any of the large particles that were common in the hand-shaking series, and on the whole the suspensions seemed more uniform. This might be expected, since there was no current to carry over the heavier particles and only those particles which were dispersed by the vibration could pass into the receiving flask.

The residue in the vibrator cup naturally varied with the sample. Of 1 gm. of soil No. 109, 0.58 gm. remained in the cup, 0.42 gm. having become suspended in the diluent. For soil No. 260, the figures were 0.38 gm. and 0.62 gm., respectively.

## MODIFIED MEDIA FOR PLATING

Preliminary tests made some years ago with Conn's asparaginate agar (*1*), variously modified media as recommended by Lipman and Brown (*4*), and soil-extract agar (*5, p. 101*), indicated that the highest and most uniform counts were obtained on soil-extract agar. This latter medium was therefore adopted in this laboratory for making routine counts of the microorganisms in the soil. The mannite-salts agar used by Whittles was originally recommended by Thornton (*10*). Three advantages were ascribed to its use—that it inhibits spreading colonies, that it is uniform in composition, and that the results can be produced with different batches of the medium. Unfortunately, no comparative results obtained with a known medium have been presented, so that its efficiency, aside from its ability to repress spreading colonies, remained in doubt. The few tests recorded in Table III were not very promising, but it seemed desirable to use this medium along with others in making routine platings of a series of soil samples taken from small squares at frequent intervals.

### COMPARISON OF MANNITE-SALTS, EGG-ALBUMEN, AND SOIL-EXTRACT AGARS

Dilutions for plating were made by adding 10 gms. of soil to 250 c. c. sterile tap water, and, after vigorous shaking for about three minutes, 10 c. c. of the suspension were added to 90 c. c. of sterile water. This was repeated until a dilution of 1: 250,000 was reached. One cubic centimeter of this dilution was then pipetted into the Petri dish, melted agar was poured in, and the plates were incubated for seven days at 28° C.

The second part of Table IV contains the results of the platings made August 13 to 27 from squares 21 to 46, inclusive, on soil-extract (S. E.) and mannite-salts agars (M. S.).[8] Four plates were poured with each medium, the figure for each square being the average of these four plates in millions of microorganisms per gram of dry soil. The counts on soil extract agar (S. E.) are from 2 to 6 times higher than those on the mannite-salts agar (M. S.), the average of the 26 tests being 30.1 and 8.0, respectively.

[8] The mannite-salts agar is made as follows: $K_2HPO_4$, 1 gm., $KNO_3$, 0.5 gm., asparagine, 0.5 gm. Dissolve in water, and add 0.2 gm. $MgSO_4$, 0.1 gm. $CaCl_2$, 0.1 gm. NaCl, and 0.002 gm., $FeCl_3$ in the form of standard solutions. Fifteen grams agar is then added and dissolved, the volume is made up to 1,000 c. c., and the agar is filtered at 100° C. through cotton. One gram of mannitol is added to the filtrate, and this is cooled to 60° C., and adjusted to P$_H$ 7.4. Sterilization in the autoclave.

TABLE IV.—*Results of frequent platings*

| Date | Mean temperature | W. H. C. | Square No. | Millions per gram | |
|---|---|---|---|---|---|
| | | | | H. | V. |
| | °F. | Per cent | | | |
| July 26 | 72 | 25 | 1 | 29.2 | 27.2 |
| | | | 2 | 27.5 | 17.4 |
| 27 | 72 | 25 | 3 | 29.5 | 27.9 |
| | | | 4 | 31.2 | 19.1 |
| 30 | 76 | 45 | 5 | 32.5 | 27.9 |
| | | | 6 | 38.5 | 25.0 |
| 31 | 69 | 53 | 7 | 39.3 | 34.4 |
| | | | 8 | 30.5 | 32.9 |
| Aug. 1 | 67 | 57 | 9 | 28.3 | 36.2 |
| | | | 10 | 28.1 | 30.3 |
| 2 | 75 | 60 | 11 | 32.9 | 29.1 |
| | | | 12 | 25.1 | 25.1 |
| 3 | 82 | 47 | 13 | 33.3 | 30.5 |
| | | | 14 | 36.5 | 33.5 |
| 4 | 82 | 50 | 15 | 30.4 | 32.6 |
| | | | 16 | 28.1 | 36.0 |
| 6 | 81 | 43 | 17 | 30.5 | 29.6 |
| | | | 18 | ----- | 30.6 |
| 7 | 84 | 43 | 19 | 26.6 | 23.6 |
| | | | 20 | 35.3 | 21.0 |
| Average | | | | 31.2 | 28.5 |
| | | | | S. E. | M. S. |
| Aug. 13 | 74 | 43 | 21 | 29.0 | 12.6 |
| | | | 22 | 31.8 | 15.5 |
| 14 | 74 | 37 | 23 | 26.7 | 11.7 |
| | | | 24 | 23.8 | 9.0 |
| 15 | 80 | 37 | 25 | 25.2 | 5.6 |
| | | | 26 | 26.5 | 6.0 |
| 16 | 73 | 37 | 27 | 24.4 | 9.8 |
| | | | 28 | 34.0 | 11.4 |
| 17 | 65 | 37 | 29 | 25.8 | 7.0 |
| | | | 30 | 21.3 | 6.5 |
| 18 | 67 | 63 | 31 | 31.5 | 7.5 |
| | | | 32 | 38.5 | 7.4 |
| 20 | 74 | 47 | 33 | 30.4 | 5.7 |
| | | | 34 | 31.5 | 8.0 |
| 21 | 76 | 47 | 35 | 30.0 | 5.8 |
| | | | 36 | 30.5 | 6.6 |
| 22 | 65 | 43 | 37 | 34.6 | 15.0 |
| | | | 38 | 31.7 | 8.2 |
| 23 | 62 | 40 | 39 | 33.9 | 7.0 |
| | | | 40 | 35.7 | 8.5 |
| 24 | 66 | 45 | 41 | 30.4 | 6.4 |
| | | | 42 | 32.0 | 4.4 |
| 25 | 70 | 47 | 43 | 29.9 | 5.1 |
| | | | 44 | 30.3 | 5.1 |
| 27 | 76 | 43 | 45 | 33.4 | 5.2 |
| | | | 46 | 30.2 | 5.2 |
| Average | | | | 30.1 | 8.0 |

| Date | Mean temperature | W. H. C. | Square No. | Millions per gram | | |
|---|---|---|---|---|---|---|
| | | | | S. E. | M. S. | E. A. |
| | °F. | Per cent | | | | |
| Aug. 28 | 75 | 33 | 47 | 26.7 | 3.0 | 20.1 |
| | | | 48 | 28.7 | 5.1 | 16.8 |
| 29 | 74 | 40 | 49 | 29.2 | 3.9 | 16.7 |
| | | | 50 | 19.7 | 4.3 | 18.5 |
| 30 | 74 | 41 | 51 | 28.6 | 5.4 | 21.9 |
| | | | 52 | 26.1 | 5.2 | 20.4 |
| 31 | 74 | 45 | 53 | 24.4 | 5.4 | 17.0 |
| | | | 54 | 29.2 | 5.7 | 22.0 |
| Sept. 1 | 73 | 40 | 55 | 16.4 | 2.3 | 17.0 |
| | | | 56 | 22.0 | 3.3 | 20.1 |
| 4 | 78 | 40 | 57 | 19.7 | 2.3 | 16.7 |
| | | | 58 | 20.0 | 1.8 | 19.0 |
| 5 | 73 | 41 | 59 | 25.7 | 5.3 | 19.5 |
| | | | 60 | 24.6 | 5.0 | 17.1 |
| 6 | 68 | 60 | 1 | 27.8 | 3.1 | 29.4 |
| | | | 2 | 24.4 | 2.1 | 27.2 |
| 7 | 74 | 60 | 3 | 27.5 | 2.2 | 25.2 |
| | | | 4 | 32.3 | 2.0 | 27.5 |
| 8 | 72 | 55 | 5 | 24.3 | 3.2 | 20.8 |
| | | | 6 | 29.7 | 4.4 | 19.0 |
| 10 | 65 | 50 | 7 | 24.0 | 3.7 | 21.7 |
| | | | 8 | 26.7 | 3.4 | 18.1 |
| 11 | 65 | 50 | 9 | 25.1 | 4.5 | 18.4 |
| | | | 10 | 21.8 | 4.4 | 17.8 |
| 12 | 71 | 48 | 11 | 26.1 | 5.4 | 20.9 |
| | | | 12 | 25.0 | 6.0 | 18.2 |
| 13 | 70 | 43 | 13 | 23.8 | 4.7 | 27.5 |
| | | | 14 | 33.7 | 5.4 | 23.6 |
| 14 | 59 | 43 | 15 | 25.1 | 8.5 | 26.8 |
| | | | 16 | 27.4 | 9.2 | 23.6 |
| 15 | 57 | 40 | 17 | 27.8 | 4.4 | 25.1 |
| | | | 18 | 21.8 | 5.8 | 17.5 |
| 17 | 58 | 37 | 19 | 30.4 | 8.1 | 25.0 |
| | | | 20 | 20.9 | 6.6 | 16.5 |
| 18 | 60 | 32 | 21 | 16.6 | 3.5 | 20.2 |
| | | | 22 | 21.7 | 3.9 | 17.8 |
| 19 | 74 | 33 | 23 | 24.4 | 5.8 | 18.1 |
| | | | 24 | 21.6 | 7.7 | 18.1 |
| Average | | | | 25.0 | 4.6 | 20.7 |
| Average | 72 | 44 | ----- | 28.0 | ----- | ----- |

* *H.*= horizontal sampling. *V.*= vertical sampling. *S. E.*= soil extract agar. *M. S.*= mannite salts agar. *E. A.*= egg albumen agar. *W. H. C.*= water-holding capacity.

In plating another series of samples (Nos. 47 to 60 and 1 to 24), taken from adjoining squares on August 28 to September 19, egg-albumen agar (E. A.) was also used. The formula of this substrate as given by Waksman (*13*) is as follows: $K_2HPO_4$ 0.5 gm., $MgSO_4$ 0.2 gm., dextrose 10 gms., $Fe_2(SO_4)_3$ trace, and agar 15 gms., dissolved in 1,000 c. c. distilled water; then 0.25 gm. egg albumen added, dissolved in 0.1 NaOH. The counts obtained on the three media are recorded in the third part of Table IV. During the progress of these tests several batches of the three media were made up and found to be uniform. The PH of each was within 0.2 of neutrality after sterilization.

Soil-extract agar again shows its superiority, while the mannite-salts agar, although a fairly good substrate for some of the soil organisms, inhibits the development of a large group of bacteria (*2*) The counts on egg albumen were consistently lower than on soil-extract agar, as Waksman (*13*) also observed. His results show that soil-extract agar gave the highest count, casein agar came next, and this was followed by egg-albumen agar. If the counts obtained by him on soil-extract agar are recorded as 100, those on casein and egg-albumen agar equal 93 and 73 per cent, respectively. Despite this decided advantage in favor of soil extract, it was recommended that egg-albumen agar be adopted for the determination of the total counts of the microorganisms in soil, and that soil-extract agar should be discarded because of its being "not standard in composition." Others have raised the objection that soil extract is of unknown composition, and that its variability would make it impossible to compare results obtained by workers experimenting with different soils. To test the validity of this objection, the following experiments were made.

### COMPARISON OF DIFFERENT SOIL-EXTRACT AGARS

Ten soils, ranging from a very rich muck to a loamy sand, were selected for preparing the extracts. It was realized at the outset that these soils presented greater differences in character than would be found in field soils likely to be used for making extracts. But since so much weight has been attached to the assumed variability of soil extracts, these writers attempted to cover wide range in one experiment. The soils used were the following, with name and source of sample, and with the soil-survey party's characterization when the sample was taken.

| | | |
|---|---|---|
| Muck | Ontario, N. Y | Very rich. |
| Peat loam | Walliston, Va | Do. |
| Greenhouse soil | Washington, D. C | Clay loam, sand, and compost. |
| Rich lawn | do | Clay loam. |
| Granby loam | Sodus, N. Y | Dark, heavy, poorly drained. |
| Genesee silty clay loam | Lyons, N. Y | Rich bottom land. |
| Silty clay loam | Arlington, Va | Field soil, low in nitrogen. |
| Hollis loam | Ludlow, Vt | Light loam, fairly fertile. |
| Dunkirk fine sandy loam | Ontario, N. Y | Light, low in humus. |
| Coloma loamy sand | Taunton, Mass | Very poor. |

The samples were all taken from cultivated fields, and air-dried. Extracts were made in a uniform manner, 500 gms. soil being boiled about 15 minutes over an open flame with 1,200 c. c. water, filtered through paper, and the volume made up to 1,000 c. c. Agar 2 per cent and $K_2HPO_4$, 0.05 per cent, were added to the extracts, and the reaction was adjusted to approximately PH 7.0.

Table V shows the results of plating 5 representative soil samples on soil-extract agars made from these 10 soil types as well as on Thornton's mannite-salts agar and on egg-albumen agar. Samples 1 and 2 were taken from a lawn (squares 25 and 26, Table IV), sample 3 came from a field at Arlington Experiment Farm, Rossyln, Va., samples 4 and 5 from two rows of pots (Nos. 34 and 35) in a greenhouse experiment. A dilution of 1:250,000 was used in plating samples 1, 2, and 3, but 1:750,000 with 4 and 5.

TABLE V.—*Influence of various soil-extract agars on the number of microorganisms growing on the plates*

| Soil-extract agar made from— | Millions per gram soil | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Average |
| Muck | 30.5 | 43.5 | 18.6 | 128 | 197 | 83.5 |
| Peat loam | 31.2 | 37.8 | 14.7 | 137 | 252 | 94.5 |
| Greenhouse soil | 30.4 | 33.9 | 14.6 | 152 | 252 | 96.6 |
| Rich lawn soil | 29.0 | 39.4 | 15.5 | 135 | 238 | 91.3 |
| Granby loam | 31.6 | 34.3 | 17.4 | 116 | 216 | 83.0 |
| Genesee silty clay loam | 33.7 | 42.8 | 19.1 | 140 | 263 | 99.7 |
| Silty clay loam | 36.8 | 44.3 | 18.0 | 149 | 260 | [a] 101.6 |
| Hollis loam | 24.9 | 33.5 | 13.7 | [a]94 | 207 | [a]74.5 |
| Dunkirk fine sandy loam | 22.6 | 31.1 | 13.5 | 143 | 220 | 86.0 |
| Coloma loamy sand | 25.2 | 32.1 | 13.1 | 122 | 233 | 85.0 |
| Egg-albumen agar | 26.7 | 34.1 | 16.2 | 66 | 181 | 65.0 |
| Mannite-salts agar | 6.7 | 16.6 | 7.4 | ---------- | 65 | 23.9 |

[a] Spreaders.

The data in Table V are very instructive. The fluctuation in the counts throughout the central part of the table is not greater than would be the case if several samples were plated on the same medium, but the appearance of the colonies on the different soil-extract agars varied considerably. Those agars containing larger amounts of organic matter were more favorable for the development of rapidly spreading colonies, especially of fungi. On the other hand, those made from light soils and containing a relatively small amount of soluble material, did not allow the development of the same number of colonies. Egg-albumen agar gave counts comparable to those on soil-extract agar when used for plating field soils, but in the case of the rich greenhouse soils its efficiency was only 50 per cent and 75 per cent. Thornton's mannite-salts agar again proved to be a rather poor substrate.

## OTHER MEDIA FOR PLATING

Conn (*1*) made total counts of bacteria in various type soils by using as plating media Lipman and Brown's "synthetic" agar, Brown's albumen agar and Fischer's soil-extract agar, in comparison with soil-extract gelatin and asparaginate agar. From his data he concluded that soil-extract gelatin and asparaginate agar[9] were the best for plating, but on account of the indefinite composition and the difficulty of handling gelatin, he declared asparaginate agar preferable. Accordingly, in plating out some samples of a field soil, the writers used soil-extract gelatin and asparaginate agar, together with a mannite-nitrate soil extract, and a "synthetic" agar. The mannite-nitrate agar was made by adding 1 per cent mannite and 0.02 per cent $KNO_3$ to soil-extract agar. The synthetic agar consisted of 2 per cent agar dissolved in distilled water containing 0.05 per cent $K_2HPO_4$, 0.1 per cent sodium asparaginate, 0.25 per cent sodium citrate, and 0.1 per cent sodium albuminate.

Table VI shows the value in these experiments, of these media for making total counts of microorganisms in the soil. Again, soil-extract agar proves superior. Although these tests are not numerous

[9] The composition of asparaginate agar was: 1,000 c.c. distilled water; 12 gms. agar; 1 gm. sodium asparaginate; 1 gm. dextrose; 0.2 gm. $MgSO_4$; 1.5 gm. $NH_4H_2PO_4$; 0.1 gm. $CaCl_2$; 0.1 gm. KCl; trace $FeCl_3$.

enough for drawing any definite conclusions, they are of interest when considered along with the others just given.

TABLE VI.—*Plate counts on different media, millions per gram of soil*

| Soil sample No. | Asparaginate agar | Soil-extract gelatin | Synthetic agar | Mannite-nitrate agar | Soil-extract agar |
|---|---|---|---|---|---|
| 1 | 7.3 | 7.0 | 11.0 | 10.0 | 18.7 |
| 2 | 3.5 | 3.3 | 10.3 | 7.6 | 10.7 |
| 3 | 4.0 | ..... | 8.1 | 8.0 | 14.4 |
| 4 | 1.5 | ..... | 5.0 | 3.0 | 9.0 |
| Average | 4.1 | 5.1 | 8.6 | 7.1 | 13.2 |

## FLUCTUATIONS IN PLATE COUNTS OF SOIL ORGANISMS

The fact that there is a seasonal rise and fall in the number of soil organisms has long been known. Daily or hourly fluctuations, however, have been studied only recently. Cutler, Crump, and Sandon (*3*) made 365 daily consecutive determinations of the bacteria and protozoa in a field soil which for a long time had received 14 tons of barnyard manure per acre annually. They observed great daily fluctuations in the numbers of bacteria, and noticed in many cases an inverse ratio between bacteria and protozoa. Soil moisture and temperature seemed to have no correlation with these fluctuations. Fortnightly averages showed the usual seasonal rise and fall and a marked parallelism in the numbers of bacteria and protozoa.

More recently the work of Cutler et al. has been continued at Rothamsted, and similar fluctuations have been observed at intervals of two hours. (*8*)

From the results obtained, it has been concluded that the usual method of making plate counts at intervals of one or several weeks does not give accurate information. Since, however, such wide fluctuations as recorded by the British investigators, as a rule, have not been recorded in bacteriological soil tests, and since, on the other hand, the fortnightly averages of those irregular counts agree closely with determinations made at longer intervals, it seemed advisable to make a series of comparative tests at frequent intervals for several weeks.

### THE EXPERIMENT PLOT AND METHODS OF SAMPLING

The soil selected for sampling was a level piece of fertile lawn, 10 feet square. The sod was removed and the earth spaded to a depth of about 6 inches. Small stakes were placed a foot apart around the edge, and strings were drawn across the plot in both directions, thus laying out 100 squares (fig. 1). In numbering them, the outside row around the entire plot, as well as the inside corner squares, were left as a border, from which no samples were taken.

The test was started two weeks after spading. The vertical and the horizontal methods were used in taking samples. The former is the usual method of taking several borings for a mixed sample. In the horizontal method, about 3 inches of the surface soil were scraped off with a clean spade or trowel, and the sample taken from the underlying layer about 2 inches in thickness and extending over an area of about 10 square inches. By careful manipulation, both

methods could be carried out on the same square. One even-numbered and one odd-numbered square were sampled each day, except Sundays and holidays, plates were poured with soil-extract agar and other media for comparison, and the colonies were counted after seven days at 28° C.

In the first part of Table IV (July 26 to August 7, squares 1 to 20), the counts of the microorganisms are given as found in samples taken by the two methods. The average for horizontal sampling is 31.2 millions, that for the vertical method 28.5 millions.

If the odd and even squares representing the opposite sides of the experimental plot are averaged separately, the same numbers are obtained with the horizontal method of sampling (31.3 and 31.2 millions per gram). But in the case of vertical sampling, the odd squares have an average of 29.8 and the even squares 27.1 millions per gram. Furthermore, the deviation of each count from the average is greater with the vertical method, the latitude being −11.1 to +7.7 millions for the vertical, and −4.6 to +8.1 millions for the horizontal.

These results indicate that greater uniformity as well as slightly higher counts are obtained when samples are taken by the horizontal method. It is well known that the greatest activity of the microorganisms occurs a few inches below the surface, and it seems preferable to take the samples from this plane without the admixture of the less densely populated soil from higher and deeper layers. In sampling with an auger it is very difficult to get equal amounts of soil from the various layers. Thoroughly mixing a soil sample is undoubtedly of some value, but it does not mix the bacteria sufficiently to assure uniform distribution of them. In view of these facts, the horizontal method alone was used throughout the remainder of the tests.

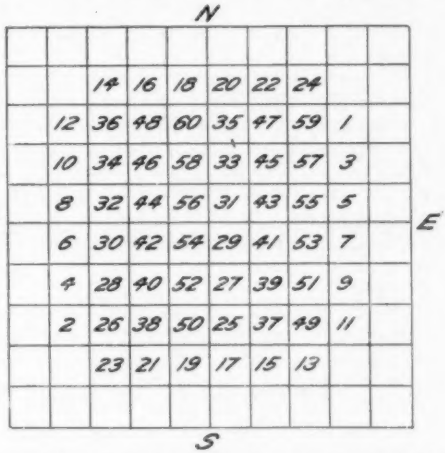



FIG. 1.—Experimental plot showing the squares in the order of sampling

### RESULTS OF FREQUENT PLATING

From July 26 to September 19, 84 squares (i. e., squares 1 to 60 and 1 to 24 inclusive) were sampled and plated on soil-extract agar and the two other substrates just discussed. The counts for each square are given in Table IV. The average for the entire series was 28 millions per gram of soil (counted on soil-extract agar). If this is taken as 100, the counts for the odd and even squares sampled on the same day exhibit certain plus or minus percentage fluctuations from the general average, as shown by the third graph of Figure 2.

The range of the fluctuations during the first half of the tests was between 25 per cent above and 15 per cent below the general average. During the second half there was a falling off in the numbers, with a fluctuation between 8 per cent above and 30 per cent below. In only two cases was the fluctuation from one determination to the next more than 25 per cent, even though one or more days had passed.

The fourth graph in Figure 2, showing the sample deviation on soil-extract agar, was made by taking the plate counts of the odd-numbered squares on each day as 100, and computing the percentage deviation of the counts of the even squares. Comparison of this graph with the preceding one shows that the variation between odd

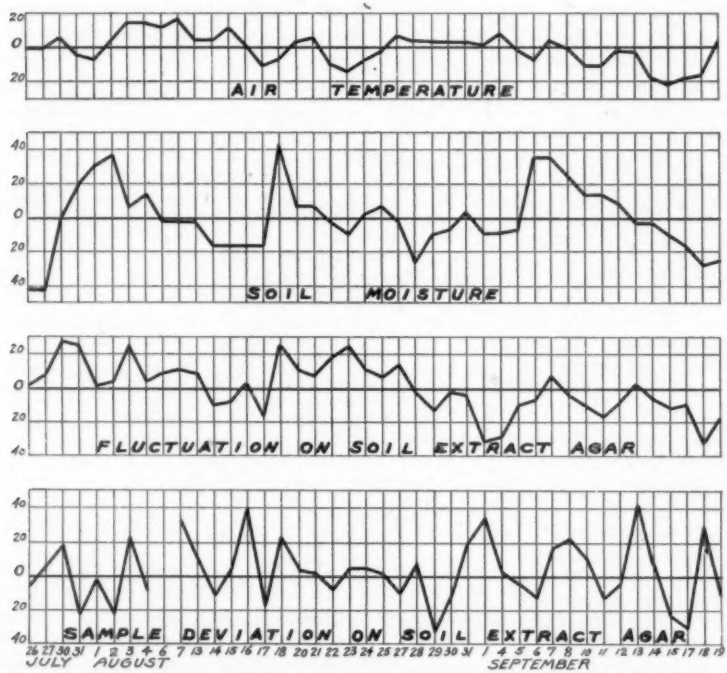



FIG. 2.—Graphic representation of plus and minus percentage fluctuations from general average of plate counts, counted on soil-extract agar

and even samples is greater than the fluctuations in the counts from one day to the next. But the averages of the odd and even squares for the entire series are practically identical, 27.9 and 28.2, respectively, showing that the experimental plot as a whole was very uniform and that the irregularities in the counts were most probably due to variation in the individual samples or unavoidable analytical errors.

The uniformity of the soil throughout the experimental plot can be further demonstrated by combining and averaging the counts from every second square across the plot, irrespective of the date of sampling (Table VII).

TABLE VII.—*Average counts of combination of squares demonstrating the uniformity of the soil*

| Squares | Average | Squares | Average |
|---|---|---|---|
| 1-47-60-36 | 27.8 | 59-35-48-12 | 27.4 |
| 3-45-58-34 | 28.6 | 57-33-46-10 | 27.1 |
| 5-43-56-32 | 30.7 | 55-31-44-8 | 27.2 |
| 7-41-54-30 | 30.0 | 53-29-42-6 | 30.2 |
| 9-39-52-28 | 30.6 | 51-27-40-4 | 30.0 |
| 11-37-50-26 | 28.4 | 49-25-38-2 | 28.4 |
| Average | 29.3 | Average | 28.4 |

The curves for air temperature and soil moisture are included in Figure 2 for comparison with the plate counts. The averages again are calculated as 100 and the daily fluctuations computed accordingly. The moisture curve shows three large fluctuations which can be likewise recognized in the plate-count curve whose gradual falling off is in general agreement with the analogous tendency of the temperature curve. In addition, slight soil differences have undoubtedly contributed to the special deviations of the plate-count curves.

As counterpart to the results obtained on soil-extract agar, curves representing the plate counts of the same samples on mannite-salts agar are shown in Figure 3. The fluctuations as well as the sample deviations are much greater on this medium, the former varying from 133 per cent above to 66 per cent below the average. Two factors may have been mainly responsible for this result—the selective character of the medium, and the smaller counts obtained, as shown in Table IV.

For comparison with these writers' results, a graph of results obtained at the Rothamsted Experiment Station has been included in Figure 3 showing the percentage fluctuations in the counts made by Cutler and his collaborators (*3*) on corresponding days of the year and using the same mannite-salts agar. It is interesting to note that the majority of the counts fall within the range of +20 per cent to −40 per cent and compare well with these present findings with the same medium, but they, too, are not as uniform as when soil-extract agar was used.

After the completion of these present experiments of the writers, Matthews (*7*), working at Rothamsted on the partial sterilization of soil by antiseptics, has reported that "the large number of counts made as controls on untreated soils failed to show, under the conditions of the experiment, any great saw-edged curve such as Mr. Cutler has proved for soils under field conditions." The counts were made in this case on gelatin plates.

### PLATE COUNTS FROM COMPOSITE SAMPLES

In making plate counts of soil organisms it is customary to make one series of dilutions, usually starting with 10 gms. of soil in 100 or several hundred cubic centimeters of water. Several plates are then poured from the highest dilution, three being probably the most common number; Thiele (*9*) recommended 20, and Waksman (*12*) used 10 plates. Other workers have used from 2 to 10 plates to get a fair average, and have usually started from one composite sample in one series of dilutions.

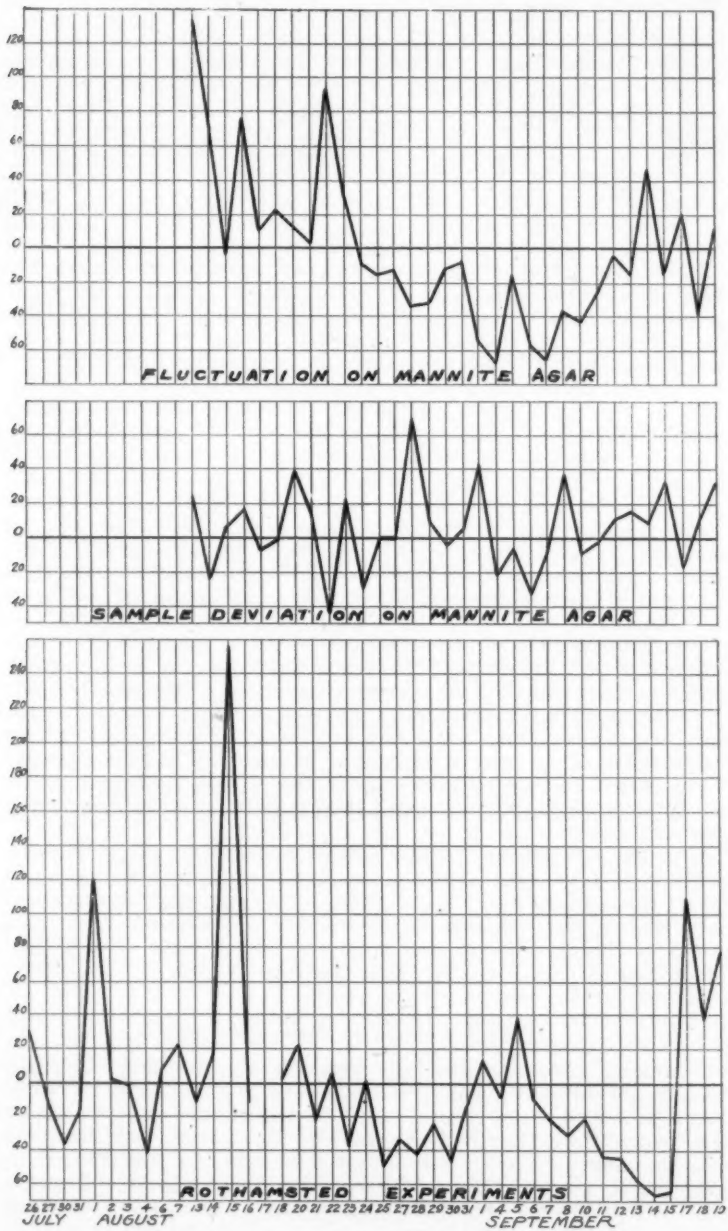



FIG. 3.—Graphic representation of plate counts of soil samples on mannite-salts agar

It remained to be seen whether duplicate series of dilutions would give concordant results. Four composite samples were taken from the plot used for the frequent platings, and two series of dilutions were made from each. From the highest dilution of each series, four plates were poured with soil extract agar and incubated for the usual seven days at 28° C. In Table VIII are given the averages of the four plates made from duplicate dilutions of four samples. The irregularity of the counts indicates that it is necessary to make parallel dilutions if a fair average is to be obtained. The same end may be attained, of course, by plating two or more samples in the usual way and taking the averages as single determinations. This is especially to be recommended if the field has been heavily fertilized and the soil is therefore of uneven structure.

TABLE VIII.—*Plate counts from duplicate dilutions of composite samples*

| Sample | Dilution | | Per cent deviation *a* from *b* |
|---|---|---|---|
| | *a* | *b* | |
| 1 | 54.0 | 60.5 | −11 |
| 2 | 104.0 | 80.5 | +22 |
| 3 | 73.5 | 78.0 | −6 |
| 4 | 120.0 | 91.0 | +24 |

From a plot ($\frac{1}{20}$ acre) which had received each year 320 pounds sodium nitrate per acre in addition to minerals, Waksman (*12*) took 51 single samples at the same time. The counts varied from 8.6 to 22.6 millions per gram (average 12.9 millions), which result illustrated the well-known fact that one plating of a single sample is very unreliable. On the other hand, 5 composite samples consisting of 3 borings, each taken from a check plot which had not been fertilized for 14 years, gave very consistent counts, ranging from 7.39 to 9.07 millions (average 8.35). The close agreement of these counts, and the wide variation of those made on the single samples, should not be attributed to the method of sampling alone, as the different treatment of the plots has evidently influenced the results obtained.

That plate counts made from a very uniform soil may show fluctuations from the average of about 20 per cent plus or minus, even though composite samples are used, may be seen from the following test. In a greenhouse experiment, corn was raised in buckets filled with 25 pounds of an evenly mixed unfertilized soil. The average dry weights recorded for eight rows, each containing eight buckets, together with the plus and minus percentage deviations from the average, as given in Table IX, clearly show the uniformity of the soil.

TABLE IX.—*Fluctuations in plate counts of uniform greenhouse soil*

| Row No. | 5 | 9 | 11 | 17 | 23 | 27 | 29 | 35 |
|---|---|---|---|---|---|---|---|---|
| Dry weight, gm. | 13.0 | 13.3 | 13.4 | 13.5 | 13.1 | 12.8 | 13.1 | 13.0 |
| Per cent ± average | −1.5 | +0.8 | +1.5 | +2.3 | −0.8 | −3.0 | −0.8 | −1.5 |
| Total counts, millions | 75.0 | 68.0 | 62.0 | 75.5 | 53.0 | 68.5 | 59.5 | 64.0 |
| Per cent ± average | +14.0 | +4.0 | −7.0 | +14.5 | −19.0 | +4.0 | −9.0 | −4.0 |

From these rows, composite soil samples were taken, and plates were poured on soil-extract agar from one series of dilutions. The counts showed the usual fluctuations from plus 14 per cent to minus 19 per cent, but no relation to the percentage variation of the dry matter of the crop. If each component of the composite samples had been plated and the average calculated on such a basis, undoubtedly the variation of the counts would have been much less, but the work involved made such a procedure impracticable.

## SUMMARY

The suspension of the bacteria in a soil sample may be effected by means of an electric vibrator of high frequency.

Plate counts made from such a suspension and from a suspension made by hand shaking gave concordant results.

Higher numbers obtained by the vibrator method would seem to indicate either incomplete sterilization of the vibrator or incomplete dispersion by hand shaking.

The greater difficulty of manipulating the vibrator precludes its use as a routine procedure.

Plates poured with soil-extract agar gave higher and more uniform results than those poured with other media. Egg-albumen agar gave lower counts, but was in general comparable to soil-extract agar. Mannite-salts agar proved to be too selective, and gave, accordingly, too low counts. Spreading colonies were equally rare on all these media.

Asparaginate agar, synthetic agar, soil-extract gelatin, and other media were all inferior to soil-extract agar.

Plate counts on soil-extract agars made from type soils ranging from loamy sand to muck showed that field soils of fair fertility, irrespective of their general character and location, are suitable for making soil-extract agars to be used in estimating the total number of soil microorganisms.

Plate counts of soil samples taken horizontally from a level approximately 4 inches below the surface are slightly higher and more uniform than those of samples taken vertically with an auger.

Fluctuations of 20 per cent above or below the average total count may occur with a uniform soil, if one series of dilutions is made and soil-extract agar used as plating medium. A more selective medium, such as mannite-salts agar, gave much wider fluctuations.

Duplicate series of dilutions of a composite soil sample showed similar fluctuations. Therefore, to be of any value, a total count should represent the average of three or more separate counts.

## LITERATURE CITED

(1) Conn, H. J.
1916. CULTURE MEDIA FOR USE IN THE PLATE METHOD OF COUNTING SOIL BACTERIA. Centbl. Bakt. (II) 44: 719–734.

(2) ———
1921. AN ABUNDANT BUT LITTLE KNOWN GROUP OF SOIL BACTERIA. (Author's abstract) Abs. Bact. 5: 8–9.

(3) Cutler, D. W., Crump, L. M., and Sandon, H.
1923. A QUANTITATIVE INVESTIGATION OF THE BACTERIAL AND PROTOZOAN POPULATION OF THE SOIL, WITH AN ACCOUNT OF THE PROTOZOAN FAUNA. Phil. Trans. Roy. Soc. London (B) 211: 317–350, illus.

(4) LIPMAN, J. G., and BROWN, P. E.
1909. MEDIA FOR THE QUANTITATIVE ESTIMATION OF SOIL BACTERIA. Centbl. Bakt. (II) 25 : 447–454.
(5) LÖHNIS, F.
1913. LABORATORY METHODS IN AGRICULTURAL BACTERIOLOGY. Tr. by W. Stevenson and J. H. Smith. 136 p., illus. London.
(6) ——— and FRED, E. B.
1923. TEXTBOOK OF AGRICULTURAL BACTERIOLOGY. 283 p., illus. New York. [etc.]
(7) MATTHEWS, A.
1924. PARTIAL STERILISATION OF SOIL BY ANTISEPTICS. Jour. Agr. Sci. 14: 1–57, illus.
(8) RUSSELL, E. J.
1923. THE MICRO-ORGANISMS OF THE SOIL. 188 p., illus. London, New York. [etc.]
(9) THIELE, R.
1903. BEITRÄGE ZUR METHODIK DER BODENFORSCHUNG. I. DIE BESTIMMUNG DER ZAHL DER MIKROORGANISMEN IM BODEN. Centbl. Bakt. (II) 11: 251–255
(10) THORNTON, H. G.
1922. ON THE DEVELOPMENT OF A STANDARDISED AGAR MEDIUM FOR COUNTING SOIL BACTERIA, WITH ESPECIAL REGARD TO THE REPRESSION OF SPREADING COLONIES. Ann. Appl. Biol. 9: 241–274, illus.
(11) ———
1923. ON THE VIBRATION METHOD OF OBTAINING A SUSPENSION OF THE BACTERIA IN A SOIL SAMPLE, DEVELOPED BY C. L. WHITTLES. Jour. Agr. Sci. 13: 352–353.
(12) WAKSMAN, S. A.
1922. MICROBIOLOGICAL ANALYSIS OF SOIL AS AN INDEX OF SOIL FERTILITY: I. THE MATHEMATICAL INTERPRETATION OF NUMBERS OF MICROÖRGANISMS IN THE SOIL. Soil Sci. (1921) 14: 81–101.
(13) ———
1922. MICROBIOLOGICAL ANALYSIS OF SOIL AS AN INDEX OF SOIL FERTILITY: II. METHODS OF THE STUDY OF NUMBERS OF MICROÖRGANISMS IN THE SOIL. Soil Sci. (1921) 14: 283–298.
(14) WHITTLES, C. L.
1923. THE DETERMINATION OF THE NUMBER OF BACTERIA IN SOIL. (Preliminary communication) Jour. Agr. Sci. 13: 18–48, illus.

# CLADOSPORIUM LEAF MOLD OF TOMATO: FRUIT INVASION AND SEED TRANSMISSION[1]

By MAX W. GARDNER[2]

*Associate in Botany, Purdue University Agricultural Experiment Station*

## INTRODUCTION

In a fall crop of Bonny Best greenhouse tomatoes at La Fayette, Ind., leaf mold caused by *Cladosporium fulvum* Cke. occurred in epiphytotic form in 1923 and produced a very prevalent and destructive, blackened, stem-end infection of the fruits of all ages. Opportunity was afforded to make a study of a large number of these infected fruits showing a wide range of symptoms.

The leaf-mold fungus has not been generally supposed to invade the fruit, although Plowright (*11*)[3] in 1887 described and illustrated a fruit rot attributed to this fungus; Halsted (*4*) reported a rot resulting from inoculations with this fungus; and, according to Makemson (*8, p. 317*), Ferraris reported that it caused a scab disease of the young fruits. The symptoms observed in this outbreak at the Indiana station did not in any way resemble the scattered lesions described by Plowright or the scab type of lesion. In fact Plowright's description of stem stripes and his picture of the fruit lesions would indicate that the mosaic disease was present in the greenhouse from which he received the specimens. Nor is there any likelihood that this rot is related to that attributed by Plowright (*10*) and Smith (*15*) to *Cladosporium lycopersici*, inasmuch as that was a blossom-end-rot infection.

Makemson (*8, p. 317*), who made a study of this disease in Michigan greenhouses, found that all parts of the blossom, including the ovaries and young fruits up to the size of a pea, were infected. He reported blasting of the flowers by this fungus, and one case of a fruit one-fourth grown which bore conidiophores and spores. However he was unable to infect fruits by wound inoculation.

Other stem-end rots of tomato have been described. The stem-end rot reported by Dickson (*1*) does not resemble the Cladosporium rot in color and was caused by a Botrytis species. A dry, black, stem-end rot of green field-grown tomatoes in Virginia, resembling the one under consideration, but caused by *Phytophthora infestans*, was described and illustrated by Reed (*12, p. 8*) and by Fromme and Thomas (*3, p. 8*).

## SYMPTOMS OF FRUIT INFECTION

The symptom which first attracted attention to this infection at the Indiana station was a sharply delimited jet black circular discoloration on the stem end of the young green fruits (pl. 1, A).

[1] Received for publication Sept. 9, 1924; issued October, 1925. Contribution from the Department of Botany, Purdue University Agricultural Experiment Station, La Fayette, Ind.

[2] The writer wishes to acknowledge his indebtedness to Prof. H. S. Jackson for helpful suggestions.

[3] Reference is made by number (italic) to "Literature cited," p. 539.

The surface of such fruits was smooth and unbroken, and at first there was no change in elevation (pl. 1, C). Later, the blackened areas tended to become more or less sunken (pl. 1, B). Soon a variety of manifestations of this peculiar stem-end rot was observed, the one constant feature being that it was always on the stem end of the fruit.

On the majority of the fruits the infected area extended rather irregularly away from the torus, usually farther on one side (pl. 1, E), and often the lesion seemed to be caused by more or less separate invasions radiating from the torus. This was very apparent in early stages of the invasion when a few small blackened areas were found extending out from the torus. In some cases a rather large lesion extended out only from one side of the torus, and on some fruits the discoloration first appeared as a slightly sunken collar or circular row of separate lesions centered around the stem end at a distance of 1½ to 2 cm (pl. 1, D). Sixteen out of 210 fruits examined showed this type of infection. Ordinarily the lesion did not involve more than one-third of the surface of the fruit, and no fruits with surface totally blackened were found.

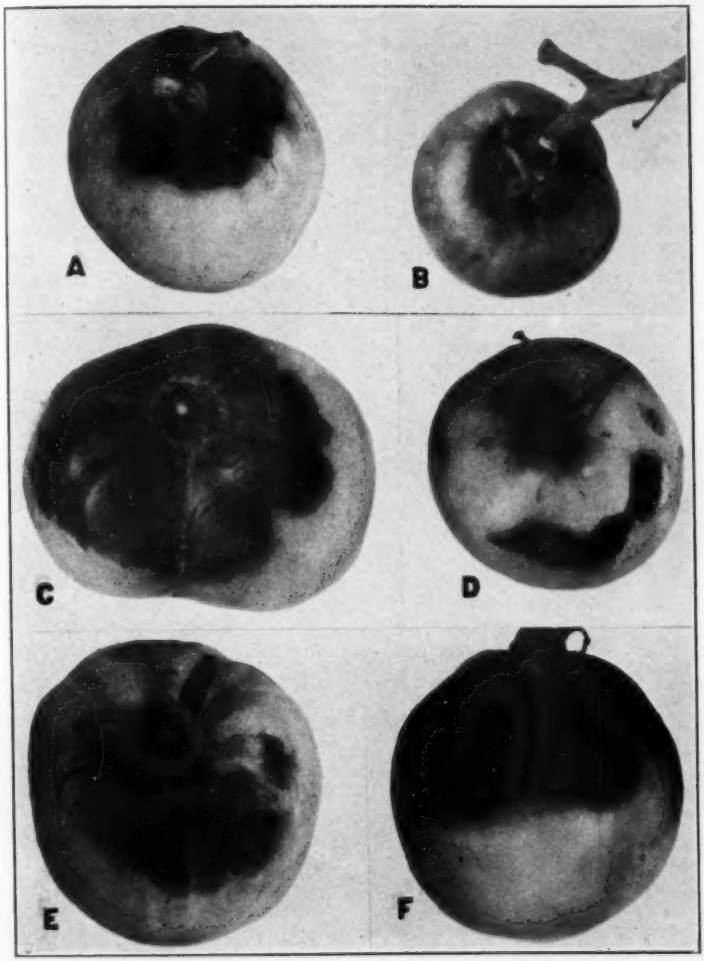
Owing to the radiating type of growth of the lesion, the margin tended to be irregularly lobed (pl. 1, A, C). Sometimes the margin was sharply defined, but frequently it was rather feathery (pl. 1, E) or the dark color blended rather gradually into the color of the apparently healthy part of the tomato (pl. 1, F).

The lesions were usually distinctly black, the color being more or less solid on the green fruits, while on red fruits it could often be seen that it was due to a blackened subepidermal network (pl. 3, A). In the former case the margin was either sharp or fading, in the latter case it was feathery or fringed. In addition to the black discoloration there were two other conspicuous color reactions. Very frequently rather small, whitish, papery patches, due to air under the epidermis, occurred on the blackened areas (pl. 2, C); and bright yellow sunken areas occurred occasionally on red fruits, probably owing to an inhibition of red pigment formation.

In the older lesions black raised subepidermal dots, very suggestive of pycnidia, occurred rather generally and were especially conspicuous in the whitish patches and yellow areas. Occasionally there were noticed on a fruit narrow, wedge-shaped, whitish or light green superficial strips radiating out from the torus, with their narrow ends toward the torus, and with scattered black dots on them.

Upon cutting longitudinally through the blackened stem-end lesions it was found that the entire thickness of the pericarp, as well as considerable portions of the locule walls and fleshy placental tissues, were involved in the blackening (pl. 2, D). Typically, there was no softening and very little structural disintegration of the affected tissues, but on the contrary these tissues were of a toughened spongy consistency. In some cases the blackened tissue could be torn loose and removed intact from the less coherent normal tissues. In other words, the affected tissues were somewhat mummified. Exceptions to this general condition were cases of secondary invasion of the killed tissues by bacteria.

Usually the internal blackening had penetrated somewhat further down into the placentae than in the pericarp or outer wall and was more extensive on the side showing the more external blackening.




A.—Jet black, stem-end discoloration of green tomato fruit, caused by *Cladosporium fulvum* Cke. Margin is sharply defined. Infection occurs through stomata in the sepal, torus, or pedicel, and the fungus eventually grows down into the fruit

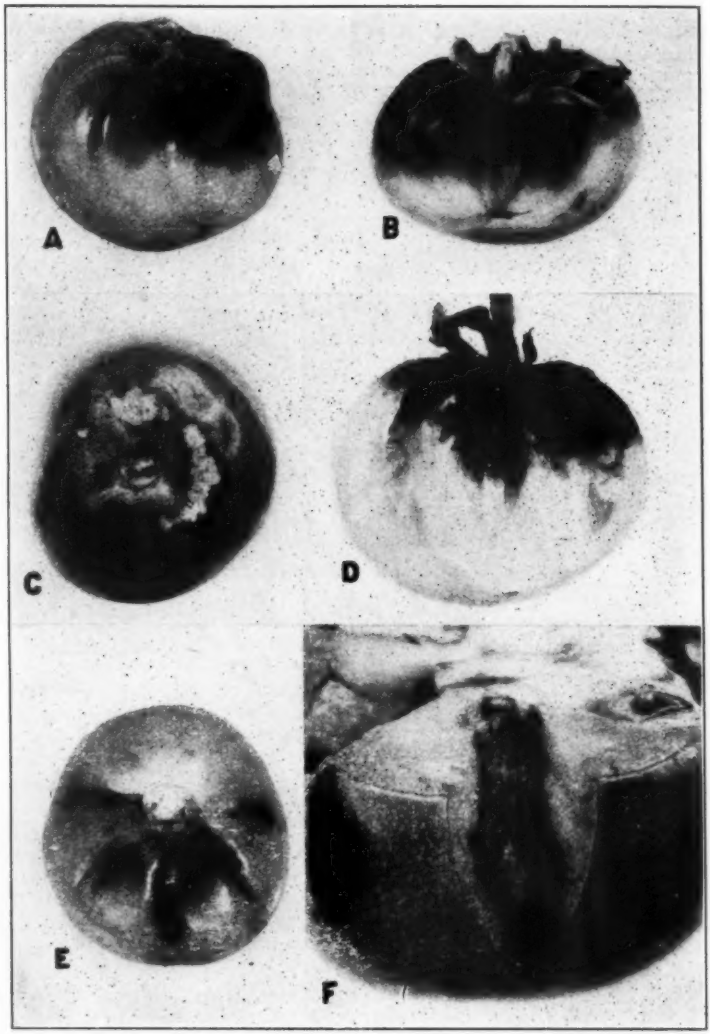
B.—Sunken, black, stem-end lesion on young tomato fruit

C.—Extensive blackening of the stem end of a green tomato. Margin sharply defined

D.—Collar of black lesions about the shoulder of the fruit more or less equidistant from the stem end. There is also some blackening about the torus. The mycelium can be traced back from such lesions to the torus

E.—Blackened lesion at one side of the stem end showing black dots under the epidermis and a feathery margin. The original infection probably occurred on one of the sepals on the side now showing the fruit discoloration

F.—Blackening of the stem end of a red fruit. The margin of the lesion is diffuse




A.—The type of asymmetry, or lopsidedness, produced by early infection of sepals and torus by *Cladosporium fulvum*. Radial furrows traverse the stunted side of the fruit, and the later invasion of the fruit by the fungus has produced a blackened area about the stem end on the stunted side

B.—Lopsided fruit as a result of sepal infection. Radial furrows traverse the stunted side. The atrophy is due probably to the early presence of the fungus in that side of the torus. Later the fungus has grown down into the fruit, blackening the tissues

C.—Stem end of an infected fruit showing whitish, papery lesions bearing stromatic or sclerotial bodies of the fungus. The white appearance is caused by air under the epidermis. The stromatic bodies may push through the epidermis in these papery spots and bear tufts of sporophores

D.—Longitudinal section showing the blackening of the stem-end region invaded by the fungus. This fruit is slightly one-sided and the most extensive blackening is in the stunted side. Placental tissues and seeds, as well as pericarp, are involved

E.—Blackened radial furrows emanating from the stem end, caused by Cladosporium invasion. One furrow shows a short transverse crack

F.—Green tomato which showed very little external discoloration cut open to show an infected placenta. The basal part of the placenta and the young ovules were atrophied and killed and contained the abundant intercellular mycellum of the fungus. ×3

Seeds in the affected region were dark brown in color. In both pericarp and placentae there was, in certain of the green fruits, some advance invasion of the blackening visible along the vascular bundles beyond the generally discolored region.

Dissection of the less blackened fruits, such as those showing a collar of black areas (pl. 1, D), disclosed the fact that the vascular bundles in the pericarp connecting these surface lesions with the torus were darkened. Upon removal of the torus it was found that usually there was a distinct darkening of the bundles in the scar, often more pronounced on the side showing the more extensive discoloration below.

There were other symptoms rather frequently associated with the disease in which the normal form of the fruit was considerably altered. One type of infection was represented by blackened furrows radiating from the stem end, in which the discoloration of the pericarp was rather deep seated (pl. 2, E). Short transverse cracks in these furrows were common, and it seemed evident that both the cracks and the furrows were attributable to a localized retardation of the growth of the fruit tissues. Dissection of such fruits showed that the dark color of the furrow was due to blackened tissue surrounding an underlying vascular bundle.

The affected fruits generally were somewhat asymmetrical or one-sided, the retarded side showing the most discoloration. This lopsidedness sometimes was very extreme, in which case the atrophied side was usually traversed by a few folds or furrows radiating from the torus toward the stylar scar (pl. 2, A, B). Upon cutting certain of these fruits which showed rather slight external blackening, the startling discovery was made that the stem end of the placentae in some of the locules was brown and atrophied, and bore small dark bodies which were found to be stunted, infected ovules (pl. 2, F).

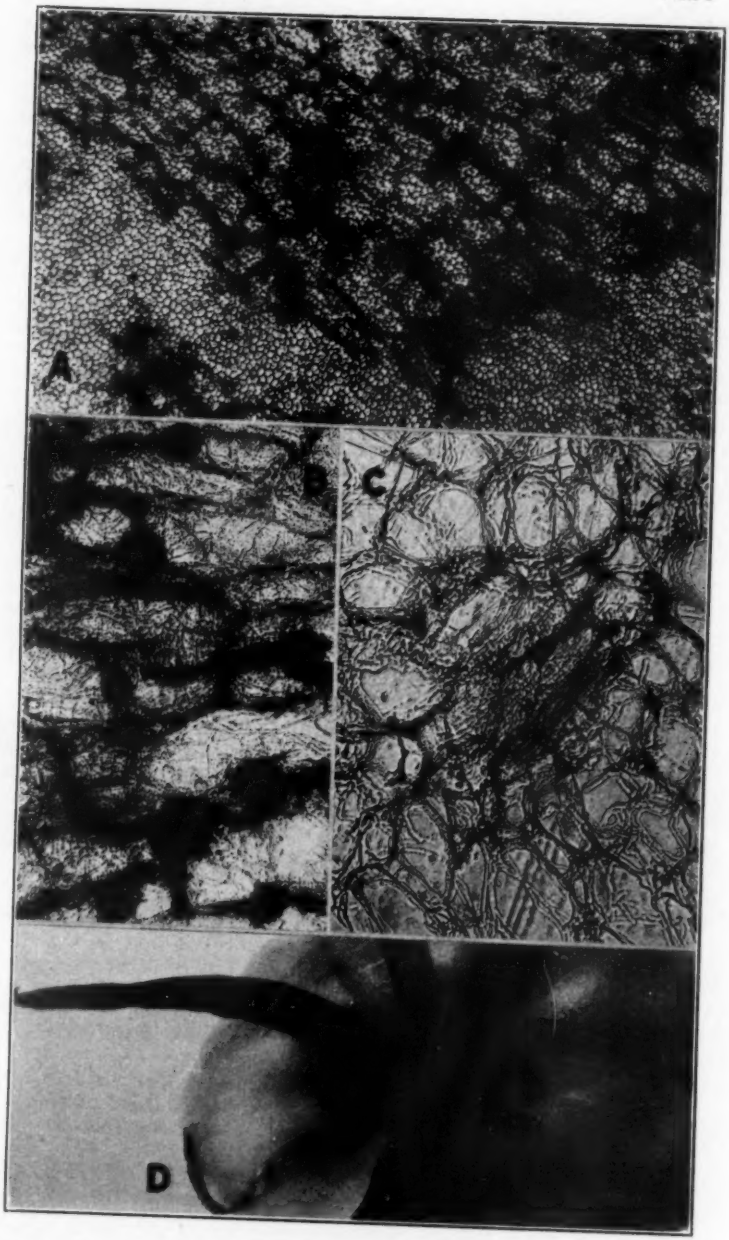
Once the significance of this lopsided condition of the fruits was appreciated, it was noted that many fruits of this character showed little or no discoloration, other than a tendency for the atrophied side to be yellowish rather than green or red. Many of such fruits developed the black color upon incubation, always more pronounced on the atrophied side, and other evidence was obtained (to be presented later) which indicated that this lopsided condition might be due to the retardation of growth on one side by this fungus under consideration.

It must be admitted, however, that this lopsidedness is not unlike that illustrated by Munson (*9, p. 49*) and attributed by him to incomplete pollination, an explanation which has been corroborated by Fink (*2*) and by later workers. It would seem, therefore, that the lopsidedness of tomatoes in the greenhouse may be due to more than one cause; in fact, mosaic necrosis and atrophy of a locule may also cause this condition.

Another striking symptom was the tenacity of the diseased fruits on the pedicels. Generally, the affected fruits were harder to remove from the plant than the normal fruits, and they did not fall off.

## THE CAUSAL FUNGUS

An examination of unstained, free-hand sections of the darkened tissues revealed the presence of dense aggregates of coarse brown mycelium in the intercellular spaces (pl. 3, A, B, C; pl. 4). From the




(For explanatory legend see p. 825)

region of advance of the stem-end blackening in the interior of three small green fruits, portions of the tissue were cut out with a flamed scalpel and planted in poured plates of 2 per cent dextrose potato agar. From 22 of the 24 plantings a similar Cladosporium developed which, in cultural and spore characters, closely resembled the leaf-mold fungus, *Cladosporium fulvum* Cke. While the destructive effects of this fungus were very evident on the foliage of this crop, it was not suspected until these isolations were made that it was the cause of the fruit rot. The fungus was also isolated from the leaves by plates poured from a spore suspension, and it seemed to be culturally identical with the form isolated from the fruit.

To prove the identity of the cultures isolated from the fruit, five young tomato plants were sprayed with a spore suspension prepared from one of the cultures, and in 15 days typical yellowed leaf lesions appeared which, 5 days later, showed sporulation. Later a second series of five plants was similarly inoculated with spores from a culture isolated from another fruit; and leaf lesions appeared in 17 days, and sporulation 5 days later. The two series of control plants sprayed with water were not infected, and the disease was not observed to be present at any time on other plants in the greenhouse. These results prove beyond question that it was the leaf-mold fungus in the blackened fruit tissues. Some of the tissue plantings had yielded bacteria as well as the fungus, indicating that the latter may be closely followed by secondary invaders in the diseased tissues.

There was no sporulation of the fungus on the fruits, except in certain restricted locations, namely, about the margin of the torus scar, along radial epidermal cracks, and in the whitened papery spots already described (pl. 2, C). The sporophores were usually borne in dense tufts on rather conspicuous stromatic bodies, the black dots in the fruit lesions previously mentioned. In the papery spots, these stromatic bodies had burst through the epidermis and were densely covered with sporophores (pl. 4, B), but in general these bodies did not break through the fruit epidermis. Agar plates were poured from the spores produced on the torus scar on an infected fruit, and the colonies were identical with isolations from a leaf and from the interior fruit tissue.

Within the tissues of the fruit, the tendency of the fungus was to fill the intercellular spaces with composite strands of brown, thick-walled, geniculate mycelium, with short, swollen cells (pl. 3, B, C; pl. 4, D, E). The individual hyphae become undulating or geniculate, each eventually bearing numerous short lateral knobs or projections which dovetail and interlock with those of adjacent hyphae to form a pseu-

EXPLANATORY LEGEND FOR PLATE 3

A.—Surface view of epidermis over a fruit lesion (such as that in Pl. 1, E), showing the blackened subepidermal network produced by the mycelium between the large parenchyma cells under the epidermis, and a few scattered sclerotial or stromatic bodies—the black dots visible to the unaided eye. Photomicrograph × 41. (All photomicrographs were made from unstained, free-hand sections of fresh material, unless otherwise stated)

B.—Tangential section of pericarp just underneath epidermis, showing the dense intercellular aggregates of brown mycelium and the spherical, intercellular, sclerotial bodies. Photomicrograph × 94

C.—Section passing obliquely through a fibrovascular bundle in the pericarp tissue. Intercellular mycelium producing somewhat of a reticulum and forming denser aggregates between the parenchyma cells immediately surrounding the bundle,which is a characteristic behavior of the mycelium in the host tissues. Photomicrograph × 94

D.—Calyx with one sepal (lower left) killed by early Cladosporium infection. The fruit was somewhat lopsided, the stunted side being under this sepal. Mycelium had penetrated into the fruit. Somewhat enlarged

doparenchyma. Makemson (*8, p. 321*) noted this type of stroma formation in his cultures on corn kernels, and it seems to be of common occurrence in culture.

In the larger intercellular spaces in the fruit tissue, not only immediately under the epidermis but throughout the interior tissues as well, this production of a pseudoparenchyma by the fungus reaches its maximum, and results in the formation of more or less spherical, stromatic, or sclerotial bodies just mentioned (pl. 3, B; pl. 4, D, E). Eventually these bodies become spherical and peritheciumlike, having an outer wall and oily contents, and measuring from 150 to 300 microns in diameter. Makemson (*8, p. 323*) noted the smaller stromatic bodies in the leaves, and found the larger bodies in his cultures on corn kernels. He (*8, p. 320*) describes the latter as "spherical bodies ranging from 50 to 120 microns in diameter" presenting "a structure typically perithecial in appearance with a thin, pseudoparenchymatous wall." These bodies, he states, were partially hollow but "contained neither asci nor spores, and could not be made to form spores by ordinary variations in the cultural technique."

In a culture on an autoclaved apple twig resting on moist cotton in the bottom of a test tube, these sclerotial bodies had developed in abundance on the cotton fibers at the end of 39 days. They had a dense dark brown wall and oily contents, and were accompanied by an abundance of short-celled, thick-walled, brown mycelium. These sclerotial or peritheciumlike bodies resemble in many ways the bulbils of certain fungi described and illustrated by Hotson (*5*). The formation of such bodies is apparently not uncommon among other Cladosporium species. Humphrey (*6, p. 228*) described hyphal knots formed by *Cladosporium cucumerinum* E. and A. in cucumber leaves, and Keitt (*7, p. 13*) found sclerotioid bodies formed by *C. carpophilum* Thum.

Cross sections of a tomato-leaf lesion near the midrib show that the fungus forms similar, but much smaller, mycelial clumps in the intercellular spaces of the mesophyll and in the substomatal chambers (pl. 4, A). Those in the latter position project or grow out through the stomata and bear tufts of sporophores, as Makemson (*8, p. 323*) has reported. A similar intercellular accumulation of mycelium occurs in the sepals, pedicels, and torus, in fact in all tissues in which the collapse and death of the host cells does not occur very soon after invasion.

EXPLANATORY LEGEND FOR PLATE 4

A.—Cross section of a leaf lesion of *Cladosporium fulvum*, showing dense intercellular aggregates of mycelium in the mesophyll and tufts of sporophores borne on mycelial aggregates or stromatic bodies in the stomata. The greater production of sporophores occurs on the lower epidermis. Photomicrograph × 110

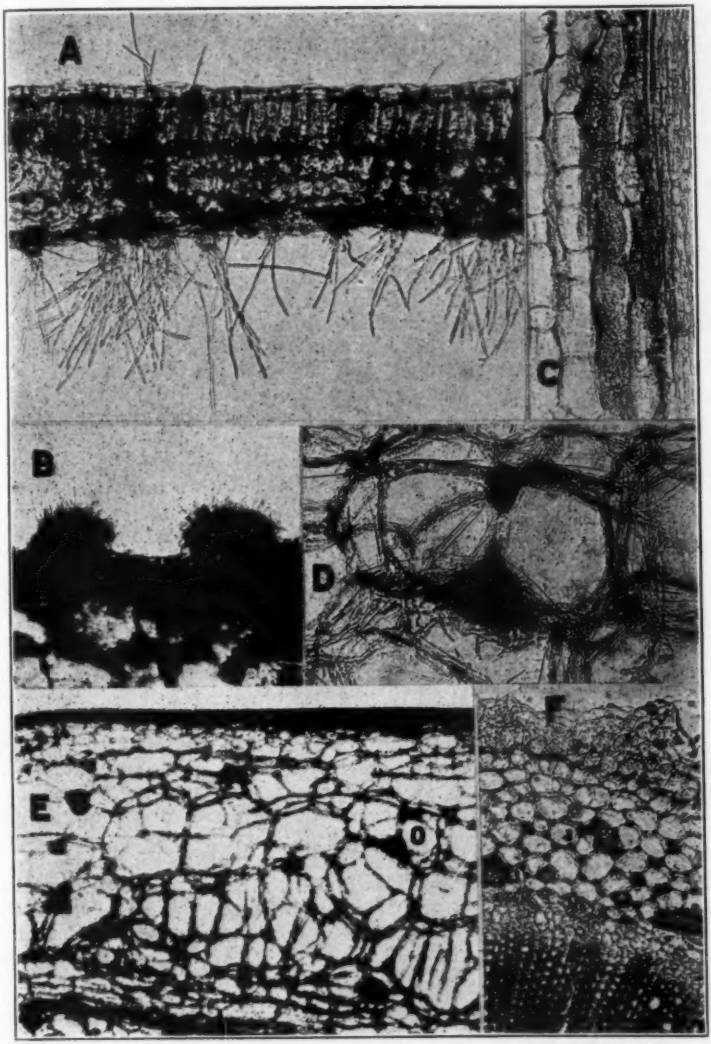
B.—Stromatic bodies covered with short sporophores. These bodies have pushed through the epidermis of the fruit in one of the white, papery lesions such as shown in Plate 2, C. In general the bodies remain subepidermal in the fruit lesions. Photomicrograph × 88

C.—Longitudinal section through cortex of an infected pedicel showing dense aggregates of mycelium between the cortical parenchyma cells adjacent to the vascular tissue (phloem) at the right. Photomicrograph × 88

D.—Enlarged view of the region marked O in the pericarp section in E, showing the multiple strands of mycelium between the cells and an intercellular sclerotial body 160 μ in diameter. Photomicrograph × 108

E.—Cross section through outer portion of pericarp showing intercellular network or reticulum of composite mycelial strands. Tissue is so thoroughly infested that it is somewhat mummified, the cavities in the reticulum representing the original host cells. Scattered sclerotial bodies occur at various points, apparently in the larger intercellular spaces. The body at O is shown enlarged in D. Photomicrograph × 35

F.—Cross section of an infected pedicel 3 mm. above the torus, showing dense masses of mycelium between the parenchyma cells of inner half of cortex, illustrating tendency of this fungus to accumulate near, but not within, the vascular tissues. Photomicrograph × 96




(For explanatory legend see p. 526)

The ultimate function of these bodies has not been ascertained. Those that are exposed to the air serve as bases of sporophore tufts. They no doubt also constitute a resistant resting stage of the fungus. It was hoped that they might develop into perithecia, but no asci have been found. These bodies developed in abundance in a large number of infected fruits held about four weeks in a refrigerator, but all remained sterile. The infected stem regions were cut from these fruits, dried a month, and then incubated in sterilized, damp sand at temperatures of 10°, 18°, 22°, 27°, and 31° C. No ascus stage developed. However, the sclerotial bodies in the dried sepals and under the white papery spots on the fruit produced sporophores and spores in abundance. Therefore, it seems that these bodies may function as a resting stage resistant to desiccation and to other organisms. But it is not at all unlikely that other parts of the brown mycelial aggregates may function similarly.

The relation of temperature to the growth of the mycelium on potato dextrose agar was determined by inoculating the center of each of eight poured plates with a loopful of a spore suspension from a strain isolated from leaves, incubating these plates four days at 25° C., measuring the colonies, and then placing the plates in damp chambers in constant-temperature incubators. The increase in colony area based on the increase in average radius during 13 days was as follows: 0 sq. mm. at 2°, 8 at 10°, 25 at 16°, 55 at 20°, 52 at 24°, 21 at 27°, 23 at 30°, and 0 at 35° C. Sporulation occurred at all temperatures except 2° and 35° C. The optimum temperature for growth was 20° to 24°, exactly as Makemson (*8, p. 328*) found in his tests. He also found that 24° was the optimum for growth and sporulation in the living leaves. In a fruit held at room temperature, the black surface discoloration about the stem end increased 4 mm. in radius in three days. In general it may be said that the fungus grows rather slowly in culture.

The relation of temperature to spore germination was determined by placing the spores in a drop of sterile tap water or prune decoction on a flamed slide in a Petri dish damp chamber and incubating at temperatures of 10°, 16°, 20°, 25°, 30°, and 35° C. A high percentage (50 to 80 per cent) of germination occurred in water in 20 hours at 20°, 23°, and 30°, and a low percentage at 10°, 16°, and 35°. The germ tubes were short in all cases, but at 43 hours were very long at 20° and 25°. At 43 hours there was 80 per cent germination at 16°. Germination seemed as vigorous in water as in prune decoction. These results indicate that the optimum temperatures for spore germination represent a slightly wider range than do those for mycelial growth. Makemson (*8, p. 329*) found similar temperatures (18° and 24°) optimum for germination in hanging drops of water, and noted in infection experiments that long branched hyphae were formed after 36 hours.

## RELATION OF FUNGUS TO HOST TISSUE

The location and distribution of the fungus in the host tissues was ascertained by examination of unstained free-hand sections of fresh material. The blackened discoloration of the fruit tissue was found to be due to the fact that the intercellular spaces were literally packed with dense aggregates of dark brown geniculate mycelium (pl. 3,

A, B). This mycelium so completely and thoroughly permeated the tissue that unstained free-hand sections gave the appearance, under the microscope, of a coarse reticulum of composite mycelial strands (pl. 4, E). This condition occurred throughout the blackened stem-end tissues, including pericarp, locule walls, and placentae. The mycelium was found considerably in advance of the margin of the darkened tissue, and here the intercellular strands were composed of fewer hyphae, more or less colorless, with longer cells of more uniform diameter.

The host tissues are not immediately killed by the intercellular mycelium, and in fact the tissues of certain organs such as the torus and pedicel and even young fruits remain alive many weeks after invasion. In fact, the fungus seems to establish a rather nicely balanced parasitic relationship with its host. In the fruits, however, the ultimate tendency of the fungus was to mummify the tissues by the formation of the heavy intercellular reticulum in which the lumina are the host cells. Thus the affected tissue gradually attains a tough, spongy character. The invaded tissues shrink or collapse very little because the original shape of the cells is preserved by the fungus reticulum.

The hyphal aggregates were always denser and larger in the large-celled parenchyma surrounding the vascular bundles (pl. 3, C; pl. 4, C, F), but apparently the mycelium does not invade the bundle tissues proper, possibly because of the comparatively small intercellular spaces. This tendency of the mycelium to accumulate in the parenchyma around the bundles accounts for the darkened bundles in the torus scar and radiating out through the pericarp and placental tissues. In its tissue invasion the fungus apparently progresses most rapidly along these particular intercellular channels. In leaf lesions resulting from inoculations, a blackened network due to this accumulation of the mycelium about the smaller veins was noted around the margin of the killed central tissues. Makemson (*8, p. 323*) also observed that the fungus in the leaves was most abundant around the tracheal tubes.

The black network noted in many of the fruit lesions was due to the hyphal aggregates between the larger parenchyma cells of the pericarp immediately under the epidermis (pl. 3, A, B). The dark radial furrows noted along the junctures of locule wall and pericarp in many fruits (pl. 2, E) were found to be due to similar aggregates of mycelium in the vicinity of underlying vascular bundles.

The fungus appears to be strictly intercellular (pl. 4, E). Hyphae were not found penetrating the host cells, even in the advanced stages of mummification of the tissue. In young fruits the growth of infected tissue and the tissues supplied by infected vascular bundles may be retarded or inhibited, and to this is attributed the furrows or depressions and the one-sided growth of the fruit. In cases of the latter showing no dark discoloration the mycelium was usually found present to a greater or less extent under the torus scar, particularly on the stunted side. In fact, in all types of fruit infection the mycelium was always to be found under the torus scar, and its distribution could be traced outward from that point. The brown, stunted, placental region at the stem end of certain fruits (pl. 2, F) was found to be entirely mummified by the intercellular aggregates of mycelium, and the young ovules were also invaded and killed.

Furthermore, as will be described later, the fungus invariably was found in the torus of infected fruits, a fact which may afford explanation for the observed failure of infected fruits to abscise normally. When infected fruits were picked, the torus usually remained firmly attached to the fruit and the break occurred at the pedicel node above the torus.

A study of a large number of cases of sepal, torus, and pedicel infection showed that the mycelium was intercellular in these organs, and, as in the fruit, formed dense aggregates in the parenchyma immediately adjacent to the fibro-vascular bundles, although none occurred within the bundles proper (pl. 4, C, F). The mycelium completely permeated the cortex, pith, and interfascicular parenchyma of the torus, but in the pedicel it was much more abundant in the cortex than in the pith. In general, the mycelial aggregates were not as dark as in the fruit and the dark stromatic bodies occurred less commonly. The distal portions of infected sepals were killed, but the tissues of the pedicel and torus apparently were not killed by the parasite, and infection of the latter organs could be detected only by microscopic examination for the presence of the mycelium, although tufts of sporophores were often present on large stromatic bodies in the stomata. Sporulation was abundant on infected sepals, especially on the lower surface, and the stromatic bodies bearing the sporophore tufts were well developed in the stomata of the sepals.

## MODE OF FRUIT INFECTION

Halsted (*4*) reported successful wound infection with spores taken from leaves but did not prove the identity of the fungus in the fruit. He also reported one case of successful infection of the stem end of the fruit. Makemson (*8, p. 322*) proved that leaf infection by *Cladosporium fulvum* occurs through the stomata, but he was unable to obtain infection of fruits, wounded or unwounded. He observed infection of all parts of the flower, and states that fruits the size of a pea and smaller were unquestionably infected. This he explains (*8, p. 319*) on the basis that the young ovary possesses stomata and lenticels which may afford an entrance to the parasite and that these stomata are rapidly transformed into lenticels, so that by the time the fruit is as large as a pea no more infection can occur. But careful microscopic examination has revealed neither stomata nor lenticels in the epidermis of young tomato fruits and ovaries, although stomata have been found on the style, torus, sepals, and pedicel.

All attempts to produce infection by artificial inoculation of fruits in the laboratory gave negative or inconclusive results. Inoculation by puncturing fruits with a flamed scalpel dipped in a spore suspension, and by placing a drop of a spore suspension about the lower margin of the torus or on the stem-end scar from which the torus was freshly removed resulted in no infection.

The best clue to the mode of fruit infection was afforded by the distribution of the mycelium within the tissues. The outstanding feature of the disease was the constant association of the lesions with the stem end of the fruit. In the few cases among the several hundred fruits examined where the lesion did not visibly emanate from the stem-end scar, the mycelium could be traced back from the visible lesion along the fibro-vascular bundles of the pericarp to the stem

end by examination of free-hand sections. As previously stated, in all types of fruit symptoms the mycelium, if present, could at least be found under the torus scar, and could usually be traced outward from that region. This held true in entirety also for the encircling collar lesions and the dark furrows, while in the cases of the greenish or yellow area on one side and the asymmetrical, lopsided fruits the mycelium had not always grown very far from the scar.

Furthermore, it was discovered by study of free-hand razor sections that in all observed types of fruit symptoms the mycelium was invariably present in the torus, a fact which is considered highly significant. If infection occurred directly through some natural wound in the stem end of the fruit, such as a growth crack or the margin of the stem-end scar which is exposed as the enlarging fruit bursts the epidermal union between ovary and torus, it would not seem that the torus itself would invariably be invaded.

Not only was the torus infested with mycelium, but frequently certain of the sepals were invaded, and the mycelium was usually present in the pedicel as far as the first node above the torus (fig. 1, N) but not beyond that point. In a number of cases carefully studied, it was found that the infected sepal was directly above the point where the fruit invasion had progressed farthest from the point of stem attachment, or was above the yellowed or stunted side of the fruit. In certain cases the mycelium in the torus and pedicel occurred only in the side towards the infected sepal, or was more abundant in that side. In one case where there were two adjacent infected sepals, the most extensive development of the lesion on the fruit was on the same side.

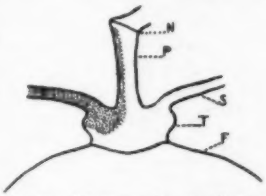



FIG. 1.—Diagrammatic longitudinal section of the pedicel node (N) and last internode (P), sepal bases (S), torus (T), and stem end of a young tomato fruit (F), showing by means of the shaded area the distribution of the mycelium of *Cladosporium fulvum* in one case observed.

In the case illustrated by Figure 1 none of the other sepals was infected, and this is regarded as an instance of sepal infection and subsequent mycelial growth down into the torus and up through the cortex of one side of the pedicel to the node. As a rule the torus eventually becomes completely permeated with the mycelium and the fruit tissue is invaded. The mycelium in the sepal or one side of the torus may retard the growth of that side of the fruit.

In one instance there were five darkened furrows in the fruit, and the five sepals above these furrows were infected, the central sepal most heavily, while the sixth sepal over the sound side of the fruit was not infected. In a case of an apparently normal fruit the mycelium in one sepal extended into the upper part of the torus cortex at the base of this sepal, and into the cortical parenchyma of the pedicel on that side, as shown in Figure 1. No mycelium was found in the fruit.

In another very similar case the mycelium extended to the base of the torus, and upon removal of the latter a slight darkening was visible about the bundles in the scar on the fruit. This fruit was incubated at room temperature, and in 10 days an infected zone 6 mm. broad was visible about the scar. In a third very similar case an infected zone 7 mm. broad appeared in 10 days, accompanied by a few black subepidermal stripes radiating from the torus scar.

These observations indicated that beyond doubt there was a correlation between the stem-end fruit invasion and the presence of the mycelium in the sepals, torus, and pedicel, and that the latter condition bore a causative relation to the former. The presence of stomata in the sepals, torus, and pedicel would readily permit spore infection, and it would seem very likely in the cases just cited that infection had occurred in the sepal, the mycelium proceeding from the sepal down into the torus and thence up into the pedicel and down into the fruit. In other cases examined no mycelium was seen in the sepals, indicating that spore infection probably took place on the torus or pedicel, both of which bear stomata. Apparently the mycelium can not pass the pedicel node (fig. 1, N), so infection, to reach the fruit, probably must occur below the first pedicel node above the torus.

Fourteen of the lopsided fruits, including some remaining green or yellow on the atrophied side and some showing the color retardation without any marked asymmetry of shape, were searched for mycelium, and in all but three cases a little was found directly under the stem-end scar. The torus was present on six, and in every case it contained mycelium. In freshly picked specimens, the mycelium was found in the atrophied or yellowed side of the fruit, and ordinarily was scanty, seldom permeating any considerable proportion of the affected side. Therefore, it would seem very likely that the lopsidedness and yellowing of such fruits was attributable to the early presence of the mycelium in the torus or pedicel, the region through which the water and food materials for the fruit must pass. However, the fungus does not seem to kill the tissues in the torus and pedicel.

These observations suggested that fruit invasion was probably the result of very early spore infection of the sepals, pedicel, or torus. To test this theory, a number of inflorescences were inoculated, in the greenhouse in March, by placing a loopful of a spore suspension on the uninjured pedicel, torus, sepals, or stigma. In practically no cases had the ovary more than barely begun to enlarge, and, in the cases of pedicel, torus, and sepal inoculation, these organs were first sprayed with distilled water before the spore suspension was applied. In 9 out of 37 cases of sepal inoculation, and 2 out of 6 cases of blossom inoculation on the last internode of the pedicel, the fungus was found sporulating on the sepals within three to five weeks.

An examination of razor sections of the stem end of the fruit 8 to 10 weeks after inoculation showed the typical intercellular Cladosporium mycelium in 5 of the 37 cases of sepal inoculation, 1 of the 4 cases of torus inoculation, and in 2 of the 6 cases in which the last internode of the pedicel was inoculated. No invasion occurred in the 4 cases in which the next to the last internode of the pedicel was inoculated, nor in the 5 cases of stigma inoculation. The identity of the mycelium within the fruits was proved by tissue transfers to agar. It was rather surprising to find certain fruits completely infested with the intercellular mycelium and yet showing no conspicuous discoloration of the tissues. In one case of sepal inoculation a typical, dark, stem-end rot of the fruit was noted 14 weeks after inoculation. These tests prove that fruit invasion may result from very early spore infection of the sepals, torus, or last pedicel internode.

In a number of the cases of sepal inoculation the fruit became lopsided, with the atrophied side usually longitudinally furrowed and slightly yellowish. In four cases the atrophied side was under the sepals which first exhibited Cladosporium sporulation and which were most likely the particular sepals originally infected as a result of the inoculation. In one case the fungus was sporulating on three adjacent sepals, and the side of the fruit under these sepals was atrophied and was traversed by a longitudinal furrow under each of the three sepals. Upon sectioning, however, no mycelium was found in the fruit nor the torus but was found only in these three sepals just mentioned. Somewhat similar cases have herein been described previously in connection with torus infection and lopsided fruits. Therefore it seems probable that the presence of the fungus in the sepal may have an injurious effect upon the corresponding sector of the fruit.

In six cases the mycelium was found in the cortical tissues of the last internode of the pedicel (fig. 1, P), but never above the first node above the torus (fig. 1, N), a condition previously noted in connection with pedicel infection. This indicates that the mycelium is unable to pass through this node. As the fungus is strictly intercellular and readily invades the fruit from the torus, it seems likely that the intercellular spaces are continuous throughout the sepals, last pedicel internode, torus, and fruit; while the failure of the fungus to pass upward beyond the first pedicel node would seem to indicate that the intercellular spaces were not continuous through that node. Owing to the absence of stomata in the fruit, the continuity of the intercellulars of the sepals and the fruit tissue may be of some physiological significance.

Observations made in a greenhouse crop in August, 1924, about the time the lowest clusters of fruit were mature, gave further evidence that fruit invasion results from sepal infection. Leaf mold was extremely severe on the foliage; and while no black stem-end rot was found, very abundant sepal infection (pl. 3, D) had occurred as was evidenced by the sporulation of the fungus on the sepals. The prevalence of sepal infection was determined by an examination of a large number of calyces in the lower five or six clusters. (See Table I.)

TABLE I.—*Prevalence of calyx infection in greenhouse tomatoes*

| | Number calyces examined | Total per cent infected | Percentage infected on— | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 sepal | 2 sepals | 3 sepals | 4 sepals | 5 sepals | 6 sepals |
| Ovaries less than 1 cm. diameter | 243 | 52 | 34 | 12 | 4 | 1 | 0.4 | ..... |
| Fruits more than 1 cm. diameter | 294 | 80 | 36 | 20 | 16 | 5 | 3 | 0.3 |

A very high percentage of the calyces were visibly infected, even in the case of small ovaries, and in many instances more than one sepal was infected. In view of the two to three weeks of incubation between infection and sporulation, it would appear that much of this infection had occurred during the blossoming period; and it probably is this early infection which is most likely to give the fungus time to grow down into the fruit before the latter ripens and is picked.

66850—25†——3

Under the microscope, longitudinal sections of some of the ovaries with infected sepals showed the mycelium present in the sepal base and pith of the pedicel but not within the ovary. In one case of a green fruit with an infected sepal the mycelium was traced from the edge of the sepal lesion down within the tissues of the sepal into the torus, a distance of 10 mm. In the case of one lopsided green fruit, one sepal was already killed by the fungus (pl. 3, D), and it was located directly above the stunted side of the fruit. There was no discoloration of this fruit to indicate infection, but microscopic examination revealed an abundance of Cladosporium mycelium in the fruit tissue at the stem end.

The blackened stem-end discoloration is a rather uncommon effect of this fungus in greenhouses, which may be due to the fact that ordinarily the fruits are picked before the fungus has had time to grow from the sepals into the fruit, or, if the fungus has already penetrated into the fruit, the fruit is disposed of before the conspicuous symptoms develop. The particular crop in which the fruit symptoms were so conspicuous had been very much retarded by cloudy weather.

## SEED INFECTION

The advance of the mycelium of *Cladosporium fulvum* from the stem end down through the pericarp of the fruit is paralleled by its penetration into the axile placentae (pl. 2, D). In some cases the ovules are infected and killed when very young. In cases of extreme atrophy of a locule in the lopsided fruits the ovules may never develop into seeds, even though not actually invaded by the fungus. Ordinarily, however, extensive invasion of the fruit occurs at a later stage after the seeds have developed, and the placental tissues are blackened and mummified by the abundant intercellular mycelium, while the seeds in the invaded region become darkened and many show a distinct blackening of the hilum end (pl. 5, A).

Free-hand sections of the placental tissue showed the typical mycelial aggregates between the parenchyma cells around the fibrovascular bundle extending out into the funiculus of the seed. Longitudinal sections of a seed with a blackened hilum showed that the mycelium had penetrated well within the outer integument

### EXPLANATORY LEGEND FOR PLATE 5

A.—At left: Four tomato seeds removed from the invaded stem-end region of a fruit. These are darkened and show blackening of the hilum. At right: Four noninfected seeds from same fruit. Enlarged × 1½

B.—A tomato seed infected at the hilum. The seed was incubated on moist filter paper in a damp chamber. The blackened hilum is covered with sporophores and spores of *Cladosporium fulvum*. Potted plants were infected successfully with spores from such a source. × 12

C.—Seed coat, with blackened, infected hilum, carried up on cotyledons of seedling. The fungus was producing spores on the blackened tissue in this case. Enlarged × 8. In lower right corner: Primary infection of cotyledon from seedling grown from an infected seed such as shown in B. The cotyledon is covered with sporophores and spores of *Cladosporium fulvum*. Enlarged × 3

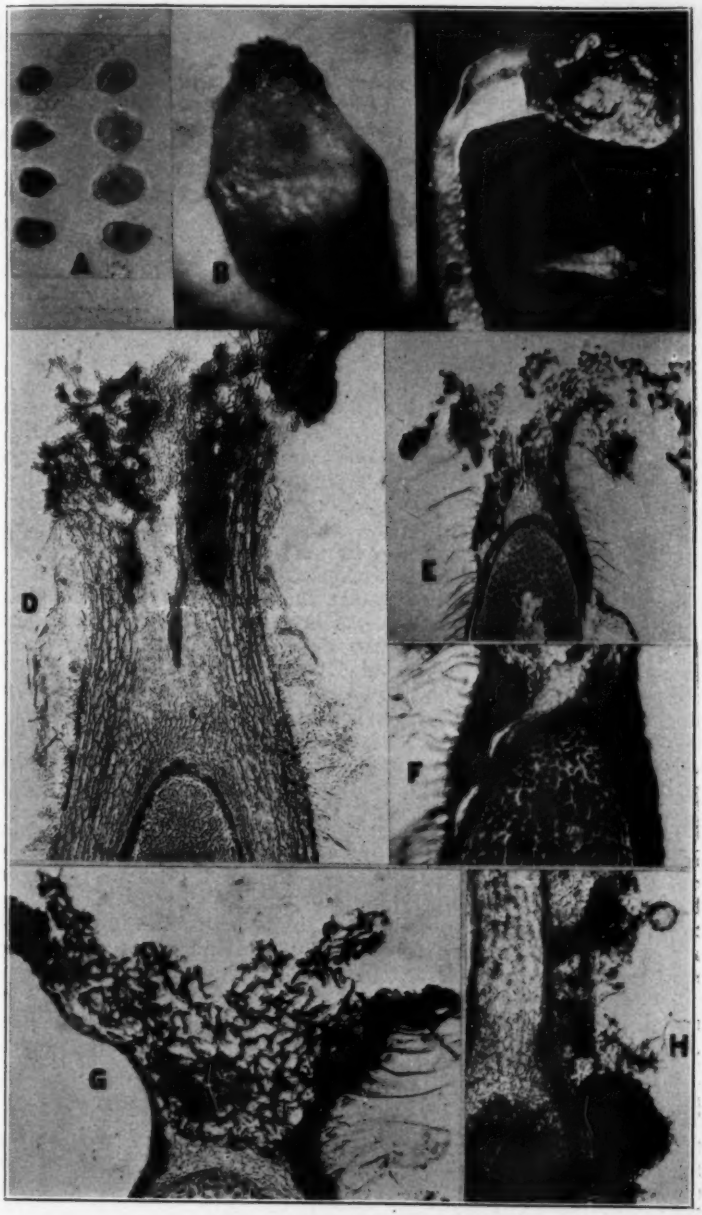
D.—Longitudinal section of the hilum end of an infected seed, showing dense aggregates of brown mycelium in parenchyma tissue of middle layer of the perisperm or seed coat. The spiral vessels of the fibrovascular bundle are shown. Part of the endosperm is seen at bottom. Photomicrograph × 96

E.—Longitudinal section of hilum end of an infected seed, showing mycelial aggregates or peritheciumlike bodies in the funicular tissue remaining attached to the seed and also crowded between the outer integument and endosperm. With collapse of the parenchyma of the middle layer of seed coat, these bodies cause the outer integument to bulge outward. No invasion of the endosperm has been noted. (Stained with carbol fuchsin). Photomicrograph × 36

F.—Mycelial aggregates crowded between outer integument and endosperm. (From a longitudinal section of same seed shown in E, stained in carbol fuchsin.) Photomicrograph × 72

G.—Longitudinal section of hilum end of infected seed, showing the intercellular mycelium in parenchyma of middle layer of the seed coat extending down almost to the endosperm, the edge of which is seen at the bottom. One peritheciumlike or sclerotial body is shown at the left. (Triple stain.) Photomicrograph × 88

H.—Mycelial aggregates attached to exterior of seed at hilum end by entanglement of the mycelium with the cellulose rods or hairs of the seed coat. Photomicrograph × 81




(For explanatory legend see p. 534)

at the hilum and had formed the usual dark-brown intercellular aggregates of mycelium in that part of the parenchyma of the middle layer of the seed coat which remains uncollapsed in the hilum region of the seed (pl. 5, D, G). These mycelial aggregates were conspicuous immediately around the fibrovascular bundle which extends from the funiculus into this tissue. Sclerotial bodies were also formed in this tissue (pl. 5, E, F, G). In certain instances observed the mycelium had invaded the parenchyma of the middle layer as far as the boundary of the endosperm (pl. 5, G), and in some cases had even grown down a short distance at the sides of the endosperm, forming dense aggregates and occasionally sclerotial bodies in this region. With the collapse of the middle layer, these bodies are crowded between the endosperm and the outer seed coat and cause the latter to bulge outward at that point (pl. 5, E, F). No mycelium was detected within the endosperm, however, probably because of the apparent absence of intercellular spaces.

In addition to the internal infection of the seeds, there was also a more or less extensive mycelial infestation of the palisade layer of long cellulose rods or hairs which cover the outer integument of the seed. The mycelium in some cases had accumulated in these hairs near the hilum and formed well developed sclerotial or peritheciumlike bodies entangled among the hairs and thus firmly attached to the seed coat (pl. 5, H). Thus it seems that the seed may be infested by the fungus externally as well as internally.

The identity of the fungus within and on the seeds was proved by incubating the latter in a moist chamber. Eleven seeds which showed infected hila and which had been dried 10 days were placed on moist filter paper in a Petri dish. Abundant sporulation of Cladosporium occurred on the blackened tissues of 6 of these seeds within 8 to 13 days (pl. 5, B). Long spore chains were produced, some containing as many as five spores. The spores were typical of *C. fulvum* and prompt germination occurred in water. In fact, many of the spores had germinated in the damp air of the Petri dish. Agar plates poured from a suspension of these spores yielded typical *C. fulvum* colonies.

Leaf infection of young tomato plants was obtained by spraying with a suspension of the spores from one of the cultures isolated from a seed. Sporulating lesions were found 23 days afterwards on all of the inoculated plants, while the check plants remained healthy. One plant was successfully inoculated by spraying the lower sides of the leaves with water and touching some of the drops with a seed coat bearing conidia about the hilum.

In one of the cases of successful sepal inoculation previously described, the fruit was examined 14 weeks after the inoculation and it was found that abundant seed infection had occurred.

## SEED TRANSMISSION

That the mycelium of *Cladosporium fulvum* may invade the seed and establish itself on and within the seed coat has been shown. In no case does it appear that the endosperm or embryo is actually invaded by the fungus. The parasite may also gain access to the seed in another way. It was found that in extracting the seed from diseased fruits fragments of the blackened, infected fruit tissue very frequently adhere to the surface of the seed, and that, after

drying, these fragments were not easily dislodged. Likewise, Cladosporium spores from infected sepals might lodge on the surface of the seed. The likelihood of seedling infection from the seed-borne fungus depends upon a number of factors, some of which have been studied.

The viability of the fungus in and on the seeds has been proved and its behavior determined by incubating the seeds on moist filter paper in damp chambers. Sporulation occurred on both the actually infected and the contaminated seeds. In some cases spores formed in abundance after four days at room temperature. Eleven seeds with infected hila were incubated at 27° C., and sporulation occurred on 6 at the end of 13 days. Thirty seeds bearing blackened tissue fragments were similarly incubated, and sporulation occurred on the blackened fragments of 3 of them after 13 days. Many of the spores germinated in the saturated atmosphere of the damp chamber.

With regard to the longevity of the fungus in and on the seeds, it should be noted that the seeds in the two tests just mentioned had been dried only 10 days, while later tests proved the fungus alive after one montn. It would seem that the dense mycelial aggregates within and upon the seed coat were excellent adaptations for the survival of long periods of desiccation. Savelli (*13*) and later Makemson (*8, p. 335*) found that the spores endured desiccation for six months and the latter found that some spores a year old were viable.

It is important to note that the moisture and temperature conditions conducive to germination of the seed are likewise conducive to sporulation as well as to spore germination, particularly if the seed lies upon the surface of the soil or if the seed coat is carried up by the cotyledons, a very common occurrence among tomato seedlings. It is to the advantage of the parasite that the infected or contaminated seed should germinate and carry the seed coat up above ground.

The germinability of the seeds bearing tissue fragments obviously will be unharmed. Of the 30 seeds of this type tested on moist filter papers as already mentioned, 27 germinated and 14 carried the testa up on the cotyledons. In one of these latter cases the fungus was sporulating on the tissue fragment. Of the seedlings grown in sand or greenhouse soil, however, a lower percentage carry up the seed coat. Of the 11 seeds with infected hila which were similarly tested, 8 germinated and one carried up its seed coat (pl. 5, C), in which case the the fungus was found sporulating on the infected tissue of the hilum. In another test, 9 out of 10 infected seeds germinated. Thus, apparently, the invasion of the seeds by *Cladosporium fulvum* does not materially reduce their germinability, and such seeds, as well as those bearing infected tissue fragments, will germinate and in certain cases carry the seed coat up into the air.

The tests just noted show that, by means of infected or contaminated seeds, the parasite might be introduced into the seed bed and be carried above ground in a sporulating condition and that spore infection of near-by seedlings might very easily occur, whether the offending seedlings were themselves infected or not.

However, in the case of hilum infection, there is ample opportunity for the seedling itself to become infected, because both radicle and cotyledons emerge from the seed coat directly through the infected region (pl. 5, C). Observation of a specific instance where a radicle, in contact with an abundance of the spores and mycelium

remained uninfected, indicates that this organ is immune to infection, probably because of its lack of stomata. But the curved hypocotyl emerging from the same opening in the end of the seed coat drags after it the two cotyledons which must slide through in direct contact with the infected tissue, thus exposing them to spore contamination (pl. 5, C).

When wetted, the spore chains on the sporophores break up at once, the spores being readily dispersed in a film of water such as is very likely to be continuous from the seed coat to the emerging cotyledons, at least at some time during their emergence. Examination of an empty seed coat showed that the fungus was sporulating along both edges of the opening through which the cotyledons had emerged.

Since in the tests just noted the seedlings could not be grown in the damp chambers long enough to obtain evidence of infection, similar plantings were made, February 7, in pots of steamed sand placed in an inclosed compartment in a greenhouse in which the disease had never been present. These pots were watered with such care that there was practically no spattering. Not very many of these seedlings carried the seed coats up into the air. An examination made after 29 days revealed no infection, but 10 days after that 2 seedlings, each of which bore an infected cotyledon, were found (pl. 5, C). One of these was among the 9 seedlings grown from seeds with infected hila, and 1 among the 40 seedlings grown from seeds bearing adherent fragments of the infected fruit tissue. These seeds had been dried one month before planting. In these cases of primary infection there was abundant sporulation of the parasite on both surfaces of the infected cotyledon, more abundant however, on the lower. From such primary infections, the spores might readily be spread by air and water to other plants in the plant bed. Thus the disease might establish itself in a crop by primary infection of the cotyledons from either infected or contaminated seeds.

It seems then that the disease may be transmitted by infected or surface-contaminated seed as a result of the sporulation of the fungus on the infected tissues. If there is any fruit infection in the fields from which commercial seed is saved, the disease may be widely disseminated through such seed. The disease occurs only to a slight extent in the field crop in Indiana; it seems to be more prevalent as a field disease in more humid regions. It has been reported as a serious field disease near Norfolk, Va., by Smith and Zimmerley (*14*); and it has been reported from South Carolina; and, according to the reports of the Federal plant disease survey, it has occurred as a field disease in Florida, Louisiana, Virginia, Kentucky, and Ohio. It would seem possible, therefore, that fruit infection may occur in seed fields. Greenhouse growers who are saving seed should guard against the selection of infected fruits.

## SUMMARY

A conspicuous, black, stem-end rot of both immature and ripe greenhouse tomatoes has been found to be caused by the leaf-mold fungus, *Cladosporium fulvum* Cke.

This fungus also causes blackened radial furrows in the fruit, and lopsided fruits which tend to remain green or yellow on the retarded side.

The mycelium is intercellulár, and the fungus produces composite strands or pseudoparenchymatous aggregates which mummify the host tissues and form a reticulum in which the cavities represent the original host cells. The mycelium is not found within the fibrovascular bundles, but completely permeates the parenchyma and tends to accumulate most extensively between the parenchyma cells immediately adjacent to the vascular bundles. Invaded tissues may remain alive for a considerable period.

Scattered throughout the affected tissues, the fungus forms, in the larger intercellular spaces, sclerotial or peritheciumlike bodies or bulbils 150 to 300 microns in diameter. No spores have been found within these bodies. Those formed under stomata in sepals, pedicel, and torus, and in certain restricted areas on the fruit lesions, may bear tufts of conidiophores.

Mycelial growth and spore germination occur between 10° and 30°, with an optimum at 20° to 25° C.

In an infected fruit the fungus occurs in the pericarp, locule walls, and placentae, invariably, it would seem, in the torus, and frequently in one or more sepals and the last internode of the pedicel.

No stomata have been found in the fruit. Histological studies and inoculation tests indicate that spore infection occurs rather early through stomata in the sepals, torus, or last pedicel internode, after which the mycelium grows down into the fruit, causing dark discoloration. The presence of infection in the sepals or torus may produce a stunting effect on one side of the fruit which results in lopsidedness of the fruit. Sepal infection is of common occurrence.

The mycelium grows down through the placentae and invades the seeds both externally and internally. Sclerotial bodies are attached to the exterior of the hilum end of the seed coat. The fungus also grows into the parenchyma tissue of the middle layer of the seed coat at the hilum, penetrating as far as the endosperm, but not into the latter. Sclerotial bodies are formed within this middle-layer tissue.

Fragments of infected fruit tissue may adhere to the seed coat of seeds extracted from diseased fruits.

Under moist conditions the fungus sporulates on infected and contaminated seeds. Since the seed coat is often carried up on the cotyledons of the seedling, the spores may readily reach other seedlings.

In germination, the cotyledons must emerge through the infected hilum region. Primary cotyledon infection occurred among seedlings grown in pots of sterile sand from both infected and surface-contaminated seed which had been dried one month.

## LITERATURE CITED

(1) Dickson, B. T.
1920. stem-end rot of greenhouse tomatoes. Phytopathology 10: 498–500, illus.

(2) Fink, B.
1894–98. pollination and reproduction of lycopersicum esculentum. Geol. and Nat. Hist. Survey Minn. Bul. (Bot. Ser. II) 9: 636–643.

(3) Fromme, F. D., and Thomas, H. E.
1916. spraying and dusting tomatoes. Va. Agr. Exp. Sta. Bul. 213, 14 p., illus.

(4) HALSTED, B. D.
1885. A TOMATO DISEASE. Proc. Soc. Prom. Agr. Sci. (1883/84) 4/5: 42–44.
(5) HOTSON, J. W.
1912. CULTURE STUDIES OF FUNGI PRODUCING BULBILS AND SIMILAR PROPAGATIVE BODIES. Proc. Amer. Acad. Arts and Sci. 48: 227–306, illus.
(6) HUMPHREY, J. E.
1893. I. DISEASES OF THE CUCUMBER PLANT. Mass. Agr. Exp. Sta. Ann. Rpt. (1892) 10: 212–231, illus.
(7) KEITT, G. W.
1917. PEACH SCAB AND ITS CONTROL. U. S. Dept. Agr. Bul. 395, 66 p., illus.
(8) MAKEMSON, W. K.
1919. THE LEAF MOLD OF TOMATOES, CAUSED BY CLADOSPORIUM FULVUM CKE. Ann. Rpt. Mich. Acad. Sci. (1918) 20: 311–348, illus.
(9) MUNSON, W. M.
1893. PRELIMINARY NOTES ON THE SECONDARY EFFECTS OF POLLINATION. Me. Agr. Exp. Sta. Ann. Rpt. 1892: 29–58, illus.
(10) PLOWRIGHT, C. B.
1881. ON THE FUNGOID DISEASES OF THE TOMATO. Gard. Chron. (n. s.) 16: 620–622, illus.
(11) ———
1887. TOMATO DISEASE (CLADOSPORIUM FULVUM). Gard. Chron. (III) 2: 532, illus.
(12) REED, H. S.
1911. TOMATO BLIGHT AND ROT IN VIRGINIA. Va. Agr. Exp. Sta. Bul. 192, 16 p., illus.
(13) SAVELLI, M.
1914. SULLO SVILUPPO DEL "CLADOSPORIUM FULVUM, VARIETAS VIOLACEUM." Ann. R. Accad. Agr. Torino 56: 63–66.
(14) SMITH, L. B., and ZIMMERLEY, H. H.
1921. RELATION OF PRESSURE TO EFFECTIVENESS IN SPRAYING TOMATOES Va. Truck Exp. Sta. Bul. 33/34: 163–190, illus.
(15) SMITH, W. G.
1887. DISEASE OF TOMATOES. BLACK SPOT (CLADOSPORIUM LYCOPERSICI). Gard. Chron. (III) 2: 408.

# FLUCTUATION IN THE DISTRIBUTION OF THE COLORADO POTATO BEETLE[1]

By C. H. BATCHELDER

*Research Assistant, Department of Entomology, Cornell University*

## INTRODUCTION

Fluctuation in the abundance and distribution of the Colorado potato beetle (*Leptinotarsa decemlineata*) is a constantly observable phenomenon in a potato-growing region. So inconspicuous does this insect become during occasional years that some growers do not feel justified in using a poison with their early sprays. Furthermore, in any year the distribution of the species in a given locality is extremely uneven. Great variation is found in the percentage of infestation from field to field, from farm to farm, and from one locality to another, in the same year as well as over a period of years. No satisfactory explanation of the phenomena has been offered except that of parasitism. Field studies, however, reveal certain variable factors operating in a northern latitude to effect variation in the abundance of the Colorado potato beetle in adjacent fields and also an important factor which influences the abundance of the insect over a series of years. It may be desirable to set down evidence of the nature of these variables as well as certain conclusions that may be considered with profit by growers.

## FIELD STUDIES

The operation of these variables was demonstrated in field studies undertaken to determine whether correlation existed between the percentage of infestation and various environmental factors. The data in this study, involving six varieties, were taken during the period August 17 to 23, 1919, a year of comparatively light infestation, in 12 townships of northern Maine, from 57 potato fields with a total of 786 acres. In contrast, data are used that were taken from the same fields, with a few exceptions, in a year of comparatively heavy infestation, during the interval August 23 to 25, 1921, from 27 fields totaling 273 acres. During the interval September 8 to 11, 1924, similar studies were undertaken in 3 counties in western New York in 28 fields totaling 226 acres. The conclusions derived from these studies have been confirmed by field observations in other northern potato-producing regions. The data taken at all fields being the same, included the following: Variety, acreage, date of planting, type of soil, fertilizer, windage, slope, sprays, disease, condition of the crop, and the percentage of the plants infested. The percentage of infestation figure was determined from field readings of 100 plants taken at random throughout the field, and was based on the presence or

[1] Received for publication Nov. 25, 1924; issued October, 1925.

absence of foliage injuries. The figure indicates, but does not represent, the degree of severity of the infestation. Tabulation and comparison of these readings indicate that the only variables affecting the percentage of infestation are earliness of appearance of the potato shoot above ground, type of ridge cultivation (covering operation), the nature of the previous crop, the application of the early sprays, and the proximity of wild or "volunteer" potato plants.

### PROTECTION OF EARLY COVERING

The "ridge" rather than the "hill" method of cultivation is followed consistently throughout the district in northern Maine from which figures were obtained. After breaking up the soil with a 4-inch cultivation, a disk hoe (occasionally a blade hoe) is used to throw the loose soil into a somewhat sharp ridge. Naturally, a deeper cultivation results in higher ridges. Ridging is begun when the potato sprouts first break through the surface soil. The sprouts are covered to a depth of 2 or more inches. The practice is then repeated as soon as the sprouts appear again, and sometimes they are covered for a third time before the rate of growth becomes too rapid to keep the sprouts covered. Ridge cultivation confers several advantages in addition to protecting the young plants from infestation. Great variation occurs in the height as well as the care with which the ridge is constructed during the covering operation as well as subsequently. In this paper it is indicated whether a low, medium, or high ridging was practiced. In general, high-ridge cultivation is correlated with more compact covering. An indication of the protection afforded by the covering operation may be seen in Table I, which gives data taken from adjacent fields where, other conditions remaining the same, both low and high ridging were practiced side by side.

TABLE I.—*High-ridge versus low-ridge cultivation*

A.—IN TYPICAL AND ADJOINING FIELDS

| Field No. | Variety | Planted | Fertilizer | Previous crop | Ridging | Per cent infested |
|---|---|---|---|---|---|---|
| 136 | Bliss Triumph | May 21 | 4-8-4 | Potatoes | High | 20 |
| 137 | do | May 18 | 4-8-4 | do | Low | 100 |

B.—AS INDICATED IN ALL FIELDS STUDIED

| Type of ridg ng | 1919 | | | | 1921 | | | |
|---|---|---|---|---|---|---|---|---|
| | Number of varieties | Number of fields | Acreage | Per cent infested | Number of varieties | Number of fields | Acreage | Per cent infested |
| Low ridging | 4 | 7 | 35 | 82.57 | 3 | 9 | 60 | 97.22 |
| Medium ridging | 6 | 27 | 445 | 36.66 | 4 | 10 | 50 | 84.70 |
| High ridging | 6 | 23 | 206 | 23.08 | 3 | 8 | 186 | 63.62 |

Ridging is not uniformly the practice in western New York, and where this method is followed it is not always done early enough to cover the shoots. Here growers cover more often for weed eradi-

cation than for insect control. Planting dates are later, and hence no protection from frost injury is conferred. It is evident, furthermore, that both the ridging and the covering operations under western New York conditions are done as the season dictates and are not standard cultivation practices. That the covering operation had a protective value during the season of 1924 in western New York is shown in Table II.

TABLE II.—*Control effect of the covering operation in western New York*

| Operation | Not covered | Covered |
|---|---|---|
| Number of varieties | 5 | 6 |
| Number of fields | 16 | 11 |
| Acreage | 143 | 82.9 |
| Per cent infested | 50.91 | 13.31 |

## INFLUENCE OF THE PREVIOUS CROP

Examination of field data also indicates that the nature of the previous crop has a marked influence on the infestation of the Colorado potato beetle. The usual rotation practice followed in the regions studied places sod a year ahead of potatoes, but when potatoes precede potatoes in the same soil a very considerable increase in the percentage of infestation has been noted. In respect to this point, the writer's figures confirm a relation already observed by keen growers. The fact that heavier infestation occurs in fields planted the second year to potatoes may be explained by the natural sluggishness of the overwintering adults on emergence in the spring. Finding the cultivated potato close at hand they accept the plant and give rise to the first brood upon it. The origin of these overwintering adults may be traced to two sources. Injured tubers left in the field after harvest attract late-emerging adults of the second brood which are obliged to accept them as food in place of foliage for a short interval before entering winter hibernation quarters. Naturally the presence of overwintering adults in soil previously planted to potatoes is also explained by infestation the previous year. The importance of this factor of the previous crop in influencing the percentage of infestation is shown in a comparison of two adjacent fields in which potatoes were growing under identical conditions, except that in one the previous crop had been potatoes and the other had been in sod (Table III).

TABLE III.—*The effect of the previous crop*

A.—IN TYPICAL AND ADJOINING FIELDS

| Field No. | Variety | Planted | Fertilizer | Ridging | Previous crop | Per cent infested |
|---|---|---|---|---|---|---|
| 2291 | Jersey Giants | May 10 | 3-8-6 | Medium | Potatoes | 87 |
| 2292 | do | do | 3-8-6 | do | Sod | 2 |
| 2176 | Irish Cobbler | do | 4-8-4 | do | Potatoes | 28 |
| 2177 | do | May 5 | 4-8-4 | do | Sod | 2 |

TABLE III.—*The effect of the previous crop*—Continued

B.—AS INDICATED IN ALL FIELDS CONSIDERED IN NORTHERN MAINE

| Previous crop | 1919 | | | | 1921 | | | |
|---|---|---|---|---|---|---|---|---|
| | Number of varieties | Number of fields | Acreage | Per cent infested | Number of varieties | Number of fields | Acreage | Per cent infested |
| Potatoes | 4 | 26 | 259 | 56.07 | 3 | 11 | 61 | 95.45 |
| Sod | 7 | 31 | 427 | 24.51 | 4 | 16 | 212 | 73.81 |

C.—AS INDICATED IN ALL FIELDS CONSIDERED IN WESTERN NEW YORK

| Previous crop | Number of varieties | Number of fields | Acreage | Per cent infested |
|---|---|---|---|---|
| Potatoes | 4 | 5 | 46.75 | 76.2 |
| Sod | 6 | 23 | 179.4 | 32.8 |

Under western New York conditions the same relation holds true (Part C, Table III).

## EARLINESS

A third variable factor influencing the percentage of infestation is the earliness with which the plants make their appearance above ground. This is usually thought of in terms of planting date, or variety, or fertility of the soil, but in any case the result is the same. If a field is planted to an early variety such as Irish Cobbler, or if a late variety is planted early or in a favorable situation where it receives a stimulus to early and rapid growth, the percentage of infestation will be very much greater. The potatoes which come up earliest are infested first, and natural dispersal to varieties appearing later is slow and usually inconsiderable except where influenced by spraying. An indication of how this factor operates in adjacent fields in which all factors remain the same except the earliness with which the plants push through the top soil is shown in Table IV.

TABLE IV.—*The influence of variety*

TYPICAL ADJOINING FIELDS OF SAME VARIETY PLANTED EARLY AND LATE

| Field No. | Variety | Acreage | Fertilizer | Ridging | Previous crop | Planted | Per cent infested |
|---|---|---|---|---|---|---|---|
| 1927 | Spaulding | | | | | | |
| | Rose | 30 | 4-8-6 | High | Sod | May 5 | 40 |
| 1933 | Spaulding | | | | | | |
| | Rose | 5 | 4-8-2 | Medium | Sod | May 20 | 20 |
| 1928 | Bliss | 20 | 4-8-6 | High | Sod | June 10 | 14 |
| | Triumph | | | | | | |
| 1930 | Irish Cobbler | 30 | 4-8-6 | High | Sod | May 10 | 65 |

Colorado potato beetles do not select early varieties exclusively, neither do they shift from late to early variety habitually. A late variety planted early will "trap-crop" for early varieties planted late, as may be noted by comparing the results in the adjoining fields Nos. 1927, 1928, and 1930.

Apparently the adult insects drop into a potato field without deliberation and are satisfied if they find potato plants. Observations made in the Solanaceae garden at Ithaca in 1923 indicate that distinction is made within the family. A common idea is current among growers that Colorado potato beetle adults manifest a preference for early varieties when offered a choice of host plants, but it would appear from variety tests that the insect does not have any particular preference when offered the opportunity of wide choice of varieties. It would seem that the insect arrives on some varieties only after trial and error, and that it does not make a distinction between varieties. This has been indicated in variety-test plots where all factors remained the same except variety.

### THE VOLUNTEER

That early spraying of potato sprouts soon after they come up is effective in reducing infestation is a common observation among growers and in the literature of the subject. In addition to the lethal effect of the spray there is a certain amount of protection afforded by the deterrent influence of the Bordeaux-arsenical sprays. Redispersal flights often closely follow spray applications. Repelled from further feeding by either the spray residues or alimentary disturbances occasioned by arsenic ingestion, the beetles take flight for a more hospitable environment. One may locate them sometimes resting on nearby foliage, as if contemplating what to do next. Such redispersal flights afford an opportunity for infestation of other fields and wild Solanaceae, the most common of which is the wild or "volunteer" potato.

The "volunteer" potato is a plant which has grown from tubers remaining overwinter in the ground or from tubers thrown away in waste areas. In a northern latitude they occur most frequently in fields sown to oats, wheat, and other similar grain crops following potatoes. Low temperatures prevent decay in the fall, an early snow blanket protects from heavy ground frost, and tubers missed during the previous fall harvest develop plants the following spring which come up with the new crop. It is not unusual for the plant to perpetuate itself; volunteers may be found in the third and sometimes the fourth year after the field was originally planted in potatoes. Great fluctuation in the numbers of volunteers per acre occurs, due to the fact that in some years the ground freezes deeply before the snow comes and the tubers are killed and "volunteers" are either very scarce or absent the following year. Dispersing adult beetles are then forced to choose between wild species of Solanum and the cultivated crop. After a deliberate hunt for infested species of wild Solanaceae, the writer is convinced that the beetles do not accept them within smelling distance of cultivated potatoes. This opinion, based on observation, is confirmed by the fact that high rates of infestation follow years favorable to the development of volunteers, such as were the years in which the data reported in this paper were taken. The importance of the volunteer potato plant as a refuge and reservoir for the potato beetle is not overdrawn. In one locality the writer counted 202 volunteers per acre in wheat, with an average infestation of 36 per cent; in wheat in another locality they ran 3,360 per acre, with an average infestation of 16 per cent, and in clover 621, with 54 per cent infestation.

The volunteers in a hay or grain field often are quite sufficient in numbers, therefore, to attract dispersing beetles, but they are not always the most important source of infestation. In grain fields where cultivation has broken up the soil, volunteers get an early start and offer hospitality to adults emerging from estivation in the same field that had been in potatoes during the preceding year. In timothy, however, the volunteer makes slow growth and is infested only late in the season. On the large plants in the grain fields the insect can pass its entire seasonal cycle without resort to the cultivated plants. The volunteers, therefore, constitute a local reservoir from which adult beetles emerging from pupation or estivation disperse to adjacent wild plants or cultivated fields. The writer is of the opinion that the insect may come from such a source

FIG. 1.—Left: "Volunteer" potato plants in buckwheat and rye. Right: Irish Cobbler field

as this when it suddenly appears in well-sprayed fields, as it often does. Occasionally the beetles migrate from defoliated volunteers in great numbers. In one case noted on August 13, 1921, a field of seedling potato plants became so heavily infested with adults from nearby volunteers that it was necessary to hand pick the field in order to save the small plants. One plant harbored 151 adults. The source of this infestation was an adjacent oat field in which the volunteers were very numerous and well developed.

In another example, rye and buckwheat were sown following potatoes, and Irish Cobblers were planted in sod immediately adjoining. The volunteers showed a 78 per cent infestation by the first brood as compared to 11 per cent in the cultivated field. Obviously the emerging adults were satisfied with the volunteers alongside of which they emerged from winter estivation as compared with the

unsprayed cultivated field of the same variety. The subsequent history was a severe infestation of the Cobbler field by the adults of the first brood, although the writer found that many of the adults had remained on the volunteers. Figure 1 is a photograph of these two fields showing their adjacent relation.

The foregoing refers specifically to conditions prevailing in northern Maine.

In western New York, sufficient data are lacking to establish definite relations between the presence of volunteers and infestation. The writer has found volunteer potato plants in wheat fields and their presence there has been noted by growers, but in the season of 1924 they were so few in number, their size so small, and their infestation so light, that no importance could be attributed to them. Whether the season of 1924 was normal in this respect was not determined.

In regard to control, the writer has noted that spraying oat fields with iron sulphate has not materially reduced the number of volunteers, and he does not at this time attempt to suggest a means for their eradication. Consistent crop protection by means of arsenicals and farm practice heretofore considered is, of course, indicated.

## SUMMARY

Although great progress has been made in the development of artificial methods for the control of the Colorado potato beetle (*Leptinotarsa decemlineata*), the insect is a persistent and expensive annual pest. Little attention has been centered on methods of protecting the potato crop other than the use of the arsenical stomach poisons.

Certain variable factors operate in a northern latitude to cause a variation from farm to farm, or from field to field, in the percentage of infestation of the beetle.

A study of infested potato fields in western New York and northern Maine indicates these variable factors to be the height of the cultivation ridge, the nature of the previous crop, the earliness in the appearance of the shoot above ground, the deterrent effect of the early sprays, and fluctuations in the numbers of volunteer potato plants.

Farm practice with these factors in mind is recommended.

# THE AMMONIA CONTENT OF SOIL, AND ITS RELATION TO TOTAL NITROGEN, NITRATES, AND SOIL REACTION [1]

By HORACE J. HARPER

*Assistant Chief in Soil Chemistry, Iowa Agricultural Experiment Station*

## INTRODUCTION

Although the ammonia content of soil has been studied by several investigators, in most cases only a few soils have been examined. Russell (*12*)[2] found that the ammonia content of unmanured soil varied from 0.5 to 1.6 parts per million; that in manured soils it varied from 4 to 7 parts per million; and that three other field soils contained from a trace to 2.2 parts per million. Matthews (*10*) reported the ammonia content of nine soils in his work on methods. The maximum amount of ammonia found was 8 parts per million, and the minimum 1.4 parts per million. Potter and Snyder (*11*) found from 7 to 25 parts of ammonia per million in seven soils. Ellis and Morison (*2*) found more ammonia in acid peats than in neutral peats, varying from 79 parts per million in the neutral peats to 296 parts per million in the acid peats. This difference was attributed to unfavorable conditions for nitrification. About 50 to 80 per cent of the ammonia in the peats could be extracted with distilled water. Jodidi (*6*), (*7*), (*8*), in his investigation of the chemical nature of the organic nitrogen in the soil, reported on the ammonia content of a few peats and on a plot of soil receiving different kinds and amounts of organic matter. The amount of ammonia varied from 50 to 440 parts per million in case of peats, and from 1.68 to 15.08 parts per million in one series of soil experiments, and from 27 to 33 parts per million in another. Kelley (*9*) found that the ammonia content of uncultivated soils varied from 2 to 42 parts per million, and that in cultivated soils the variation was from 4.2 to 33.6 parts per million.

## EXPERIMENTAL DATA

This investigation was planned to determine whether there is any relation between the ammonia content of soil, soil reaction, total nitrogen, or the amount of nitrates present. In the first experiment 24 soils were used. Five samples were obtained from Professor Truog, of Wisconsin; five from Professor Duley, of Missouri; and the others from various parts of Iowa. All samples were placed in pyrex beakers and left in a covered box in the greenhouse from March 20 until July 5. They were made up to an optimum moisture content occasionally. At three different times during the incubation period they were allowed to become quite dry, with the object of approximating the conditions in fallow plots. All of the soils were moist when the ammonia and nitrate contents were determined. Samples were dried and ground for the total nitrogen determinations.

[1] Received for publication Nov. 3, 1924; issued October, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 553.

Total nitrogen was determined by the Kjeldahl-Gunning method.

Nitrates were determined by the phenoldisulphonic acid method, using the modification as recommended by the author (*4*) in case of colored extracts.

Soil reaction was determined by the Truog test (*13*).

Ammonia was determined by the aeration method as recommended by Potter and Snyder (*11*), except that large test tubes 1½ by 14 inches were used instead of Kjeldahl flasks to hold the soil suspension, and a cotton plug was inserted between the soil suspension and the absorption bottle to stop the spray formed during aeration. Fifty grams of soil and 100 c. c. of ammonia-free water containing 5 c. c. of a 40 per cent $K_2CO_3$ solution were aerated for 8 hours at a rate of 200 liters of air per hour. A little paraffin oil was used to prevent foaming. The amount of ammonia was measured by nesslerization, except in case of the larger amounts which were determined by titration.

The results of the first experiment are given in Table I. There is evidently no correlation between the total nitrogen content of the soil and the ammonia content as Russell (*12*) suggested. Nor, evidently, is there any correlation between the ammonia content and the reaction of these soils. In practically every instance where a large amount of ammonia was present in the soil, the nitrate content was also quite large; however, in some instances the nitrate content was quite large and the ammonia content quite small.

TABLE I.—*Ammonia, nitrate, total nitrogen content, and acidity of 24 soils varying widely in physical and chemical properties*

| No. | Soil type | Source | Acidity | Total nitrogen per 2,000,000 pounds of soil | Nitrogen as nitrate | Ammonia |
|---|---|---|---|---|---|---|
| | | | | *Pounds* | *P. p. m.*[a] | *P. p. m.* |
| 1 | Raw peat | Manistique, Mich. | Very strong | 43,800 | 328.5 | 32.8 |
| 2 | Decomposed peat | Madison, Wis. | Not acid | 24,360 | 236.5 | 23.2 |
| 3 | Clyde silty clay | Racine County, Wis. | do | 9,260 | 137.6 | 7.5 |
| 4 | Webster clay loam | Ames, Iowa | do | 8,630 | 51.2 | 6.7 |
| 5 | Waukesha silt loam | La Crosse, Wis. | Strong | 6,550 | 68.2 | 3.4 |
| 6 | Shelby loam | Jamison, Iowa | Slight + | 5,210 | 45.5 | 7.8 |
| 7 | Tama silt loam | State Center, Iowa | Strong | 4,280 | 125.3 | 8.2 |
| 8 | Carrington silt loam | Springville, Iowa | do | 3,900 | 51.2 | 3.9 |
| 9 | Shelby loam | Udell, Iowa | do | 3,380 | 196.0 | 60.7 |
| 10 | Marshall silt loam | Missouri Valley, Iowa | Medium | 3,280 | 68.0 | 29.9 |
| 11 | Carrington loam | Calamus, Iowa | Strong | 3,280 | 41.6 | 3.7 |
| 12 | Baxter gravelly loam | Polk County, Iowa | Slight | 2,800 | 128.0 | 18.7 |
| 13 | Miami silt loam | Rochester, Wis. | do | 2,740 | 44.1 | 3.5 |
| 14 | Carrington loam | Independence, Iowa | Strong | 2,460 | 30.4 | 2.6 |
| 15 | Grundy silt loam | Mountain Pleasant, Iowa. | Medium + | 2,460 | 74.7 | 7.9 |
| 16 | Shelby loam | Jamison, Iowa | do | 2,240 | 90.7 | 6.0 |
| 17 | Marion silt loam | Unionville, Iowa | Very strong | 1,990 | 31.2 | 7.2 |
| 18 | Clarksville gravelly loam | Lawrence County, Mo. | Slight + | 1,700 | 64.1 | 46.6 |
| 19 | Waverly silt loam | Polk County, Mo. | do | 1,580 | 28.8 | 16.6 |
| 20 | Buckner coarse sand | Muscatine, Iowa | Strong | 1,530 | 28.1 | 4.5 |
| 21 | Marion silt loam | Boone County, Mo. | do | 1,510 | 36.1 | 9.6 |
| 22 | Knox silt loam | Missouri Valley, Iowa | Not acid | 1,370 | 12.0 | 9.1 |
| 23 | Lintonia sand | Mississippi County, Mo. | Slight + | 700 | 46.0 | 11.2 |
| 24 | Carrington sand | Vinton, Iowa | Medium + | 560 | 21.6 | 3.9 |

[a] P. p. m.=parts per million.

It may be that the ammonia content of soils is influenced quite largely by soil management. Because the treatment of the soils used in Table I was not known, a second series of soils was collected. Eleven samples were taken from plots receiving no treatment on several of the outlying experimental fields, one sample was taken from an onion field near Rudd, four samples were secured near Logan, and a sample of shale was secured near Fairfield.

The results of these analyses are given in Table II. The ammonia content was not affected by the crop, neither was it correlated with total nitrogen or acidity. Soil No. 2, which was growing a crop of onions, had a very high nitrate content; and the ammonia content also was large. The nitrate content varied with the crop grown. It was low in soils growing small grain and higher in case of soils growing legumes and corn (*1*).

TABLE II.—*Ammonia, nitrate, total nitrogen content, and acidity of several field soils growing various crops*

| Sample No. | Soil type | Source | Crop | Acidity | Total nitrogen in 6⅔ inches of soil | Nitrogen as nitrate | Ammonia |
|---|---|---|---|---|---|---|---|
| | | | | | *Pounds* | *P.p.m.*[a] | *P.p.m.* |
| 1 | Webster silt loam | Ames, Iowa | Oats | Not acid | 7,870 | 1.0 | 8.2 |
| 2 | Floyd silt loam | Rudd, Iowa | Onions | Strong | 6,460 | 153.6 | 20.6 |
| 3 | Tama silt loam | Newton, Iowa | Corn | Medium | 4,680 | 16.8 | 12.2 |
| 4 | Grundy silt loam | Mount Pleasant, Iowa. | do | Strong | 4,660 | 28.0 | 9.3 |
| 5 | do.[b] | do | do | Medium+ | 2,290 | 9.0 | 7.1 |
| 6 | do.[c] | do | do | Medium | 1,290 | 8.8 | 4.0 |
| 7 | do | Cedar, Iowa | Wheat | Strong | 3,850 | 1.6 | 5.6 |
| 8 | Marshall silt loam | Logan, Iowa | Alfalfa | Very slight | 3,430 | 8.6 | 6.7 |
| 9 | Putnam silt loam | Farmington, Iowa. | Oats | Medium | 2,700 | 8.0 | 7.9 |
| 10 | do[b] | do | do | Strong | 3,050 | 5.6 | 9.3 |
| 11 | Clinton silt loam | Keosauqua, Iowa. | do | do | 2,990 | 1.6 | 7.1 |
| 12 | Marshall silt loam | Logan, Iowa | do | Not acid | 2,900 | 12.8 | 6.0 |
| 13 | do | do | Sweet clover. | do | 2,800 | 12.8 | 16.8 |
| 14 | do | do | Rye | do | 2,740 | 4.0 | 5.3 |
| 15 | Marion silt loam | Lowell, Iowa | Corn | Medium+ | 2,680 | 24.4 | 16.8 |
| 16 | do | West Point, Iowa. | Oats | Medium | 1,880 | 2.6 | 4.3 |
| 17 | Shale | Fairfield, Iowa | None | Very strong | 500 | None. | 5.6 |

[a] P. p. m.=parts per million.
[b] Subsurface.
[c] Subsoil.

In the third experiment a study was made on the effect of temperature and moisture on the ammonia content of four soils which varied widely in reaction and total nitrogen content. Two series of each soil were made up to 25 per cent, 50 per cent, and 75 per cent saturation with distilled water and incubated for 6 weeks. One series was kept at a temperature of 30° C. and the other series at 15°. The results are given in Table III. They do not agree with those presented by Hutchinson (*5*), who found that ammonia accumulated more rapidly than nitrates at a temperature of 25° to 30°.

It was found in all cases but one that the ammonia content of these soils was lower at the end of the incubation period than at the beginning, while the nitrate content increased on incubation. In case of the Marion silt loam both the nitrates and the ammonia increased at 15° C., but at 30° the ammonia content decreased when the soils were kept at a moisture content of 50 and 75 per cent satu-

ration. This soil is very acid, having a $P_H$ value of 4.9. It also contains a large amount of easily replaceable iron, aluminum, and manganese. It is possible that in this case the nitrifying organisms are inhibited by the unfavorable conditions and that considerable amounts of ammonia can accumulate in the soil.

TABLE III.—*Effect of temperature and moisture on the ammonia and nitrate content of four soils*

| Soil type | Acidity | Total nitrogen in surface 6⅔ inches | Nitrogen as nitrate | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | At beginning | At end of incubation | | | | | |
| | | | | 25 per cent saturation | | 50 per cent saturation | | 75 per cent saturation | |
| | | | | 15° C. | 30° C. | 15° C. | 30° C. | 15° C. | 30° C. |
| | | *Pounds* | *P.p.m.*[a] | *P. p. m.* | *P. p. m.* | *P. p. m.* | *P. p. m.* | *P. p. m.* | *P. p. m.* |
| Webster clay loam | Not acid | 8,630 | 13.2 | 50.4 | 65.2 | 55.4 | 86.6 | 70.8 | 83.2 |
| Knox silt loam | do | 1,370 | 4.9 | 10.8 | 16.6 | 13.9 | 20.8 | 14.7 | 15.9 |
| Shelby loam | Strong | 3,380 | 12.5 | 45.8 | 90.1 | 56.4 | 103.5 | 78.3 | 91.2 |
| Marion silt loam | do | 1,990 | 11.2 | 19.8 | 38.9 | 22.1 | 76.8 | 28.7 | 60.2 |

| Soil type | Acidity | Ammonia content | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | At beginning | At end of incubation | | | | | |
| | | | 25 per cent saturation | | 50 per cent saturation | | 75 per cent saturation | |
| | | | 15° C. | 30° C. | 15° C. | 30° C. | 15° C. | 30° C. |
| | | *P. p. m.* | *P. p. m.* | *P. p. m.* | *P. p. m.* | *P. p. m.* | *P. p. m.* | *P. p. m.* |
| Webster clay loam | Not acid | 10.22 | 5.1 | 3.5 | 5.0 | 3.7 | 3.4 | 3.7 |
| Knox silt loam | do | 14.96 | .7 | 1.4 | .9 | 3.4 | .34 | 1.3 |
| Shelby loam | Strong | 20.19 | 5.2 | 2.7 | 3.9 | 2.4 | 3.4 | 3.0 |
| Marion silt loam | do | 67.32 | 74.8 | 67.4 | 74.8 | 41.2 | 65.9 | 41.0 |

[a] P. p. m. = parts per million.

During the past season the large amount of rain and very cool weather have kept the soils fairly cold. A large number of field soils have been examined and the ammonia content of none of them has been greater than 40 parts per million, while most of them contained less than 10 to 15 parts per million.

The accumulation of ammonia in soils evidently depends upon the rate of protein hydrolysis and the rate of nitrification, just as the rate of nitrification depends upon ammonification (*3*), except when ammonia is added to the soil, and the rate at which the nitrates are used by plants and microorganisms. For this reason a method which would determine the amount of easily hydrolyzable protein might give much more accurate information in regard to the availability of soil nitrogen than nitrification tests which are merely an equilibrium depending upon the rate of nitrate formation and consumption, and the results could be determined very quickly. Further investigation is in progress to determine whether such an analytical procedure can be developed which will correlate with crop growth.

## SUMMARY

A study was made of the relation between the ammonia content of soils, total nitrogen, nitrate content, and soil reaction. No correlation could be made when the ammonia content of a large number of soils was compared with the total nitrogen content, the accumulation of nitrates, or the soil reaction.

The ammonia content of a soil is evidently in equilibrium with the products of protein hydrolysis and nitrification, just as the nitrate content is in equilibrium with the ammonia content of the soil and the plants and microorganisms.

The ammonia content of most field soils does not exceed 20 parts per million, and the majority of soils contain less than 10 parts per million. Occasionally the ammonia content of some soils may be as high as 60 or 70 parts per million. This may be due to conditions being unfavorable for nitrification or to vigorous ammonification, or it may be the result of both of these factors operating simultaneously.


## LITERATURE CITED


(1) CONNER, S. D.
1922. NITROGEN IN RELATION TO CROP PRODUCTION IN THE MIDDLE WEST. Jour. Amer. Soc. Agron. 14: 179–182.

(2) ELLIS, J. C. B., and MORISON, C. G. T.
1916. THE AMMONIACAL NITROGEN OF PEATS AND HUMUS SOILS. Jour. Agr. Sci. 8: 1–6.

(3) GAINEY, P. L.
1917. THE SIGNIFICANCE OF NITRIFICATION AS A FACTOR IN SOIL FERTILITY. Soil Sci. 3: 399–416.

(4) HARPER, H. J.
1924. THE ACCURATE DETERMINATION OF NITRATES IN SOILS. Indus. and Engin. Chem. 16: 180–183.

(5) HUTCHINSON, C. M.
1913. REPORT OF THE IMPERIAL AGRICULTURAL BACTERIOLOGIST. Rpt. Agr. Research Inst. and Col., Pusa 1911/12: 78–83.

(6) JODIDI, S. L.
1909. ORGANIC NITROGENOUS COMPOUNDS IN PEAT SOILS. Mich. Agr. Exp. Sta. Tech. Bul. 4, 28 p.

(7) ———
1911. THE CHEMICAL NATURE OF THE ORGANIC NITROGEN IN THE SOIL. Iowa Agr. Exp. Sta. Research Bul. 1: 1–46; 3: 113–134.

(8) ———
1912. AMINO ACIDS AND ACID AMIDES AS SOURCES OF AMMONIA IN SOILS. Iowa Agr. Exp. Sta. Research Bul. 9: 327–362.

(9) KELLEY, W. P.
1915. AMMONIFICATION AND NITRIFICATION IN HAWAIIAN SOILS. Hawaii Agr. Exp. Sta. Bul. 37, 52 p.

(10) MATTHEWS, D. J.
1920. THE DETERMINATION OF AMMONIA IN SOIL. Jour. Agr. Sci. 10: 72–85, illus.

(11) POTTER, R. S., and SNYDER, R. S.
1915. THE DETERMINATION OF AMMONIA IN SOILS. Jour. Indus. and Engin. Chem. 7: 221–226.

(12) RUSSELL, E. J.
1910. THE AMMONIA IN SOILS. Jour. Agr. Sci. 3: 233–245, illus.

(13) TRUOG, E.
1920. TESTING SOILS FOR ACIDITY. Wis. Agr. Exp. Sta. Bul. 312, 24 p., illus.

# LOCALIZATION OF THE RESPONSE IN PLANTS TO RELATIVE LENGTH OF DAY AND NIGHT[1]

By W. W. GARNER, *Senior Physiologist in Charge*, and H. A. ALLARD, *Physiologist, Tobacco and Plant Nutrition Investigations, Bureau of Plant Industry, United States Department of Agriculture*

## INTRODUCTION

In earlier papers [2] it has been seen, with many species of plants, that modification of the duration of the daily illumination period seems to have definite formative effects on growth and of bringing about certain well-defined chemical changes within the plant.

As an aid in attempting to determine the manner in which these formative effects are brought about, it is of considerable interest to know the results when different parts of the plant are exposed to different periods of illumination. In other words, when two parts of the same plant are exposed to different illumination periods, will each part show the characteristic response to its particular light period, more or less independently of the action of a different light period on the other plant part? This question has already been answered in part in an earlier paper,[3] but in recent experiments the results have been considerably extended.[4]

In the earlier work, specimens of *Cosmos bipinnatus* Cav. were cut back to the first node above the cotyledons, from which two branches were allowed to grow under a daily illumination period of 16 hours. At the end of a month a vertical cardboard screen was arranged between the two branches of each plant, one branch being exposed only to the natural daylight period of winter, while the other branch was exposed to electric illumination from sunset till midnight, in addition to the natural illumination. Each branch showed the characteristic response to its illumination period—that is, the branch exposed to the short illumination period promptly flowered and developed seed, while the branch exposed to the longer light period remained vegetative to the end of the test.

## EXPERIMENTAL DATA

The tests previously reported had to do with two coordinate branches of a plant exposed to different day lengths. The present experiments deal with different portions of the primary stem exposed

---

[1] Received for publication Oct. 16, 1924; issued October, 1925.

[2] GARNER, W. W., and ALLARD, H. A. EFFECT OF THE RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. Jour. Agr. Research 18: 553-606, illus. 1920.

——— and ALLARD, H. A. FURTHER STUDIES IN PHOTOPERIODISM, THE RESPONSE OF THE PLANT TO RELATIVE LENGTH OF DAY AND NIGHT. Jour. Agr. Research 23: 871-920, illus. 1923.

———, BACON, C. W., and ALLARD, H. A. PHOTOPERIODISM IN RELATION TO HYDROGEN-ION CONCENTRATION OF THE CELL SAP AND THE CARBOHYDRATE CONTENT OF THE PLANT. Jour. Agr. Research 27: 119-156, illus. 1924.

[3] GARNER, W. W., and ALLARD, H. A. FURTHER STUDIES IN PHOTOPERIODISM, THE RESPONSE OF THE PLANT TO RELATIVE LENGTH OF DAY AND NIGHT. Jour. Agr. Research 23: 871-920, illus. 1923.

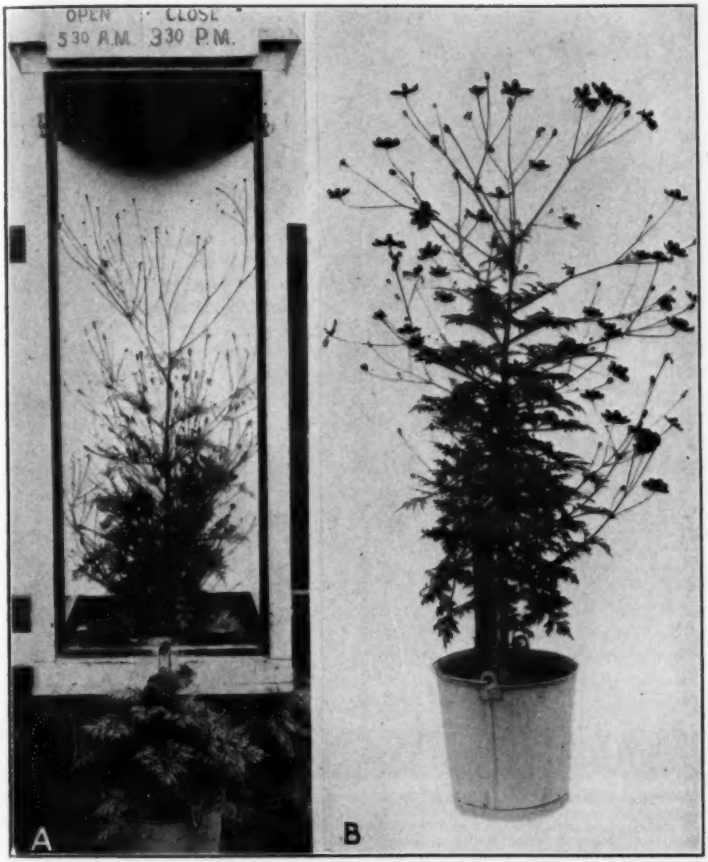
[4] In this paper and in earlier experiments dealing with plants, different parts of which have been exposed to different illumination periods, only short-day plants, flowering in response to relatively short daylight periods, have been used. Since the present paper was submitted for publication the work has been extended to a series of typical long-day plants, in which flowering is induced by exposure to relatively long daily illumination periods. Results secured to date (September 1, 1925) indicate that these plants, like the short-day plants, show localized responses, flowering only on those stems receiving a long daily illumination period. Publication of the details of these tests is deferred to a later date.

to different light periods. For these tests *Cosmos sulphureus* Cav., a typical short-day plant, has been used. When exposed to a daylight period of 12 hours or less, this species flowers readily, while it tends to remain in the vegetative stage for an indefinite period when exposed to a daylight period in excess of 13 hours (pl. 1 and pl. 2, B). Flowering normally begins at the apex and rather rapidly extends downward along the axis, numerous axillary flowering branches being developed.

The first experiment was made to ascertain the results when the lower portion of the axis is exposed to a short daily light period while the upper portion is exposed to a long one. The test was begun in the greenhouse in winter. Seedlings were grown with a daily illumination period of 16 hours till 3½ months old, electric light being used to prolong the light period after sunset. From the beginning of the experiment on January 7, the control plants were exposed to only the natural daylight, which was of about 10 hours' duration, and they began flowering February 9. Around the upper portion of the axis of the test plant a box with open front and top was so arranged that it could be raised as the plant grew taller. A 100-watt electric light placed within the box and 1 foot above the apex of the plant was turned on daily from sunset till midnight, thus giving about 16 hours of illumination daily. The front of the box about the upper part of the plant was kept covered with black cloth while the electric light was on so as to prevent the light reaching the lower part of the test plant and the controls. The upper portion of the plant inclosed in the box showed the usual response to prolonged daily illumination. It remained in the vegetative state and continued to increase in stature. The lower portion of the stem, exposed only to the short daylight period of winter, soon developed flowering branches at the nodes, the first blossom opening March 5. The appearance of the test plant and a control on March 9 is shown in Plate 2, A. Both the upper and lower portions of the axis have shown the characteristic responses to their respective light periods and the normal position of the flowering portion of the stem has been reversed.

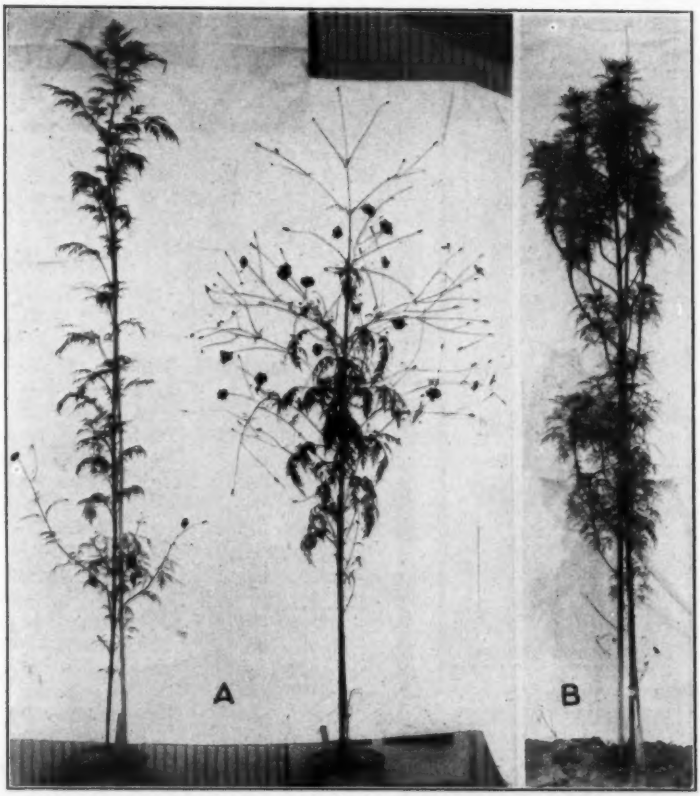
The treatment of the test plant was continued till May, when the days had become sufficiently long so that the use of the electric light could be discontinued. The plant was then transplanted outdoors. Although the entire above-ground part of the plant was exposed to the long days of summer, the lower portion continued to flower throughout the summer, while the upper portion continued its vegetative growth, as shown in Plate 2, B. By the middle of August the plant had reached a height of 11 feet. By October 1, in response to the decrease in length of day, the entire upper portion of the plant passed into the flowering condition, flower buds developing rapidly on all branches.

In this experiment and those subsequently described, as well as in the one mentioned in the opening paragraph, it would seem that any possibility of effects due to differences in water supply, plant nutrients, or other soil factors has been eliminated. It will be observed that in the earlier test the two branches growing from the same node, but differently illuminated, obtained at all times their supply of the soil solution through the same mother stem. In the present case the soil solution reaching the upper portion of the




A.—Yellow Cosmos, upper portion of which was placed in the light-proof chamber on June 13 and received thereafter only 10 hours of light daily. The lower portion of the plant received the light of the full length of day. In response to the short day, flower buds were visible in the upper portion of the plant by June 25, and the first open blossom appeared July 9. The lower portion, exposed to the long day, remained vegetative throughout the summer

B.—Sister plant of the individual shown in Plate 1, A. This plant was grown under long-day conditions until June 13, when it was given 10 hours of illumination daily. Flower buds could be seen June 28, and the first open blossom appeared July 10. Photographed July 16. This plant represents the typical behavior of yellow Cosmos when exposed to a short day. The behavior of the plant when exposed to long days is illustrated by the upper portion of the individual shown in Plate 2, B




A.—Plants from seed which germinated September 25. Seedlings were grown in a greenhouse artificially lighted from sunset till midnight each day until January 7 when test was begun. From that date control plant at right was exposed to the natural daylight period of winter, and began flowering February 9. Beginning January 7 lower portion of plant at left received the natural daylight only, while the upper portion received electric illumination from sunset till midnight in addition to daylight. In response to the short-day exposure, the lower portion showed flower buds by February 14, and the first open blossom appeared March 8. Responding to the long-day exposure, the upper portion remained in the vegetative condition and continued to increase in height. Photographed March 9

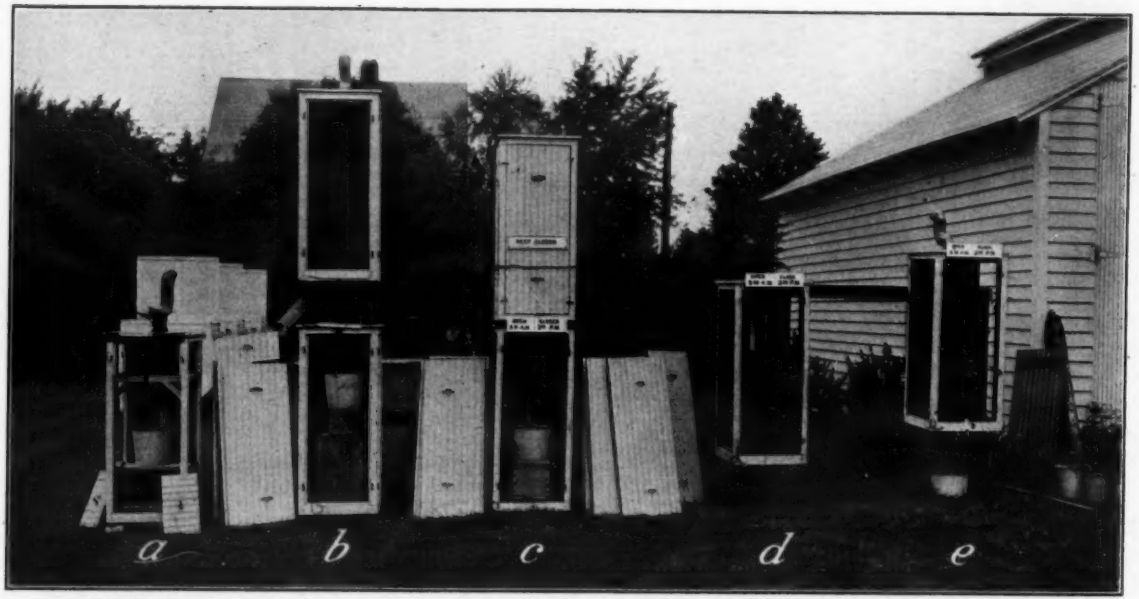
B.—Later stage of development of the Cosmos plant shown at left in 2, A. On May 7, when the natural length of day had increased sufficiently to inhibit flowering, the electric illumination was discontinued and the plant was transplanted outdoors. Vegetative development of the upper portion continued, and when photographed August 18 the plant had attained a height of 11 feet. The lower portion of plant, however, continued to flower through the summer, as shown here

primary stem, which remained in the vegetative stage throughout the exposure to long days, necessarily passed through the lower portion of the stem, which, nevertheless, continued in the flowering condition. Moreover, the same is true of the organic materials passing from the upper vegetative portion of the stem downward to the roots.

The initial experiment having given such interesting results, it seemed desirable to study further the possibilities in localizing the reproductive and vegetative phases of development in different portions of the axis in the individual plant. For this purpose a series of tests was conducted during the summer, when the days are long. To control the light exposures of the different parts of the plants light-proof boxes of suitable size, with three sides removable, were set up. Ventilation was provided for by inserting into the top and bottom or the upper and lower parts of the back of each box or compartment a 3-inch galvanized-iron elbow to which were attached two similar elbows in such way as to form a double bend. These doubly curved pipes, blackened inside and outside, proved to be very satisfactory for the purpose in view. The top or bottom of each box, as required, was provided with a slot leading to an opening in the center into which the primary stem of the test plant could be slipped and the slot then closed. A front view of the cases, showing the plants in position, is seen in Plate 3. The series of compartments here shown make possible the exposure of top and bottom of the plant axis to different day lengths, the exposure of the central portion of the axis to a day length differing from that received by the upper and lower portions, and, finally, exposure of the upper part of the axis to continuous darkness day and night while the lower portion is exposed to a regulated day length.

Seedlings of *Cosmos sulphureus* were grown during the spring months in a greenhouse in which electric light was used from sunset till midnight. Early in June the plants were set in 10-quart metal buckets and placed outdoors in preparation for the tests. As a control plant, to show the normal response to a short day, an individual was placed under a 10-hour day, beginning June 13, and the first blossom opened July 10. The appearance of the plant on July 16 is seen in Plate 1, *b*. None of the plants left in the open showed any indications of flowering till October. The upper portion of the test plant shown in Plate 2, A, may be taken as illustrating the typical behavior when exposed to a long day.

Beginning June 13, the upper portion of one of the plants was exposed to a 10-hour day while the lower portion was exposed to the full period of daylight. For this experiment the light-proof box shown in Plate 3, *e*, was used. This treatment was continued till September 23 when the plant was transferred to the greenhouse. On the upper portion of the plant exposed to the short day, flower buds could be seen June 25 and the first blossom opened July 9. No flowers or flower buds appeared on the lower portion of the stem, exposed to long days, except at the node immediately below the bottom of the box. (Pl. 1, A.) At this node, which was about 1 inch below the base of the dark box, a single blossom opened August 11. Flower buds finally appeared in the lower portion of the stem October 19, after the plant had been transferred to the greenhouse.




Series of ventilated, light-proof chambers used in regulating the duration of the daily period of illumination received by different portions of the plant. Chamber *e* is used in exposing the upper portion of plant to a short day while the lower portion is exposed to the full length of day; *d* is employed in exposing the lower portion of the plant to a short day while the upper portion receives the light for the full length of day; the two chambers, one on top of the other, *c*, are used to maintain the upper portion of the plant in continuous darkness, while the lower portion is subjected to either a long or short day, as desired; the pair of chambers *b* are arranged for exposing both the upper and lower portions of plant to a short day, while the central portion receives the light of the full day length; the chamber *a* is arranged for reversing the last-named light exposures

Beginning on the same date, another test was made in which the treatments just described were reversed, the upper portion of the plant being exposed to the full period of daylight, while the lower portion was exposed for only 10 hours daily (pl. 3 *a*, *d*). Flower buds appeared in lower portion of plant July 3, and the first blossom opened July 17. No flower buds appeared on upper part of plant until October 1 after it had been transferred to the greenhouse. Here, again, the vegetative and the flowering zones of the axis were sharply delimited by the two light treatments.

The last experiment, in conjunction with that first described, shows that flowering has been readily induced on the lower portion of the stem by exposure to a short daily light period, while flowering in the upper part of the stem was inhibited by exposure to a long daily period of illumination, whether the light was wholly natural or partly artificial and of low intensity.

Having shown that flowering was readily confined to either the upper or the lower portions of the stem by regulating the duration of the respective light periods, it was decided to study the somewhat more complex situation in which the central portion of the stem is exposed to a light period differing from that to which the upper and lower portions are subjected. Beginning July 11, the upper and lower portions of the stem of a Cosmos plant were allowed to receive only 10 hours of light daily while the central portion of the stem was exposed to the full period daylight. This was accomplished by using two light-proof cases, one arranged 12 inches above the other (pl. 3, *b*). Flower buds were visible on the lower portion of the plant July 30 and on the upper portion July 26, while the first open blossom on the lower part appeared on August 21 and on the top part on August 11. No flower buds appeared in the central zone of the stem until the plant had been transferred to the greenhouse, except that at the node immediately below the base of the upper light-proof case a few buds appeared in late August. The flower buds at this node, which was scarcely an inch below the base of the dark compartment, were unable to develop and soon perished. Each portion of the stem responded to its particular light exposure in characteristic manner, without material interference from adjoining sections responding in turn to a different light period (pl. 4, A). This experiment furnishes additional evidence that the action of a short light period in inducing flowering does not extend upward to parts of the plant simultaneously exposed to a long light period; and, similarly, this action in inducing flowering extends downward in the stem only a very short distance at most when the lower portion is exposed to a long light period.

In another test begun July 13, the treatments just described were reversed, the middle portion of the stem being subjected to a 10-hour day and the upper and lower portions being exposed to the full length of daylight (pl. 3, *a*). Flower buds were visible on September 10, on the central portion exposed to a 10-hour day, and the first blossom opened October 18. No buds appeared on the upper and lower portions exposed to the full length of day till after the plant had been transferred to the greenhouse. Because of the low height of the light-proof chamber the illumination within when the sides were open was poor and undoubtedly inadequate, and it was probably for this reason that unfolding of the flower buds was

materially delayed. In any event, the localized effect of the long and short daily illumination period is plainly evident.

Extensive data presented in the earlier papers, already referred to, make it clear that, when compared with the action of a longer daily illumination period, the effects of a shorter light period may fail to show any tendency to approach those produced by total darkness. It seems quite clear that, in general, the formative action of light on the aerial portions of the plant may readily extend to parts which are under ground and therefore excluded from light, influencing their development in various ways. That distinctive differences in the formative effects of long and short daily illumination periods may be thus transferred to subterranean organs in the case of the potato has been shown in a previous paper.[5] On the other hand, the above tests indicate that the local action of a relatively short daily illumination period in initiating flowering in yellow Cosmos is not extended to an adjoining portion of the plant which is exposed to a light period unfavorable to sexual reproduction. It seemed worth while, therefore, to determine the comparative effects of a short and a long daylight period when acting on the lower portion of the plant, in influencing the activities of the upper part exposed to continuous darkness. The first tests were carried out during the summer of 1923.

Using a combination of two light-proof chambers, one arranged immediately above the other as shown in Plate 3 *c*, the upper protion of a Cosmos plant was maintained in continuous darkness, while the lower portion was exposed to 10 hours of daylight. These treatments were begun on July 6, and on July 26 the upper portion was restored to the natural length of daylight by opening the dark chamber. During the period of darkening, the portion of the stem excluded from light increased in length from 3 inches to 21 inches. When returned to the light this part of the stem was badly etiolated, had lost the power to stand erect, and no flower buds could be seen (pl. 4, B). During the time the upper portion was darkened, the lower portion of the stem which was exposed to the 10 hours of daylight wilted badly, but this condition soon disappeared after the upper portion was restored to the light. Flower buds had appeared on the lower portion by July 18, and the first blossom opened August 11. On the upper part of the stem, which had been darkened, flower buds appeared by August 20, and the first blossom opened September 7.

It will be observed that the upper portion of the plant, restored to the full natural length of daylight after the exposure to continuous darkness for 20 days, flowered as soon as it had recovered sufficiently to do so. It appears, therefore, that the action of the short day on the lower portion of the stem was in some way transmitted to the upper portion which was darkened. These results are in line with the fact just mentioned that the formative action of light may be transmitted to parts of the plant which receive no light. This is in contrast, however, with the relations which obtain when one part of the plant is exposed to a short light period and the other to a long period. The experiment was repeated, beginning August 20 and maintaining the upper portion of the plant in continuous darkness for 28 days. The results were similar to those of the first test.

[5] GARNER, W. W., and ALLARD, H. A. FURTHER STUDIES IN PHOTOPERIODISM, THE RESPONSE OF THE PLANT TO RELATIVE LENGTH OF DAY AND NIGHT. Jour. Agr. Research 23: 891. 1923.

A.—Yellow Cosmos plant, the upper and lower portions of which were placed in light-proof chambers on July 11 and received thereafter 10 hours of light daily, while the central portion continued to receive the light of the entire summer day. Both top and bottom of the plant responded in characteristic manner to the short day and soon flowered, as shown in the photograph. The central portion, on the other hand, remained vegetative in response to the long day. Photographed September 11

B.—Yellow Cosmos plant, the upper portion of which was maintained in total darkness from July 6 to July 26 and then restored to the full length of day. While the upper portion remained in continuous darkness the lower portion was exposed to a 10-hour day. Flower buds were visible in the lower portion July 18, and the first blossom opened August 11. The upper portion was badly etiolated by the darkening, but soon recovered when restored to light and was showing flower buds by August 20 and an open blossom on September 7 in response to the supposed transmitted effect of exposure of the lower part of the plant to a short day

In the summer of 1924 further tests were conducted with yellow Cosmos. The seed germinated January 26. The preliminary treatment of the seedlings was the same as in the tests of the preceding year. The control plants remaining outdoors through the summer showed flower buds October 1. Tests involving three different light treatments were made: (1) upper portion of plant subjected to continuous darkness, and lower portion exposed to 10 hours of daylight daily; (2) upper portion exposed to continuous darkness, and lower portion allowed to receive all the daylight of summer days; (3) entire plant maintained in continuous darkness, and subsequently returned to all the natural illumination of summer days.

The first series, which was carried out to confirm the results of the experiments made the preceding year, was begun June 3. The upper portion of the plant was restored to the full length of daylight on July 9. Possibly because of the somewhat cooler weather which prevailed during this time, it was possible to prolong the exposure to darkness beyond the lengths of the exposures in the previous experiments, without irreparably damaging the darkened part of the plant. The lower part of the plant, exposed to 10 hours of daylight, showed flower buds June 28, and the first blossom opened July 15. In this instance, the upper, darkened portion likewise showed flower buds on June 28, and the first open blossom on July 15. The test was repeated, beginning July 9, and in this test it was possible to continue the exposure to total darkness for only 19 days. The lower part of the plant, in response to the 10 hours of daylight. showed flower buds July 22, and first open blossom August 8; while the upper, darkened part showed flower buds August 26, and first open blossom September 27.

The first test of the second series was begun May 31. The upper portion of the plant was restored to the full length of daylight on July 3, after having been darkened for 34 days. The lower portion of the plant, exposed to full daylight, showed no flower buds through the summer, and the same was true of the upper part which had been darkened. Flower buds finally appeared in the top of the plant at the same date as in the controls, namely, October 1. In a second test, begun July 3, the top of the plant was excluded from light for a period of 25 days while the lower portion, as previously, received throughout the test the light of the full length of day. No flower buds appeared on the plant till October 1.

In the third series, three plants were kept in darkness from July 12 to July 22. When returned to outdoor conditions, the plants were in a critical condition, due to advanced etiolation, and two of them perished. The third individual survived, turned green, and resumed vegetative development. No flower buds appeared till October 1.

## DISCUSSION OF RESULTS

These tests furnish no evidence that darkness in itself is capable of initiating flowering. Considering the whole plant, under the conditions of the test, exposure of Cosmos to continuous darkness failed to result in reproductive activity, while it has been seen that exposure to a short period of daylight seemed to promptly promote flowering and fruiting, and that exposure to a long period of daylight apparently inhibits these processes. Likewise, in the tests in localiz-

ing the action of the light period, exposure of the upper portion of the axis to continuous darkness apparently did not in itself favor formation of flower buds, for these appeared only when the lower portion of the axis was exposed to daylight for 10-hour periods, and failed to form when the lower portion was exposed to a long day. On the other hand, mere absence of light seemingly does not inhibit the laying down of flower buds in response to the transmitted influence of a short daily light period acting on an adjoining portion of the axis. A short daily illumination period may exert the same local effect in initiating sexual reproduction, without material interference from the action of total darkness or of a long daily illumination period on other parts of the plant, as when acting upon the green portion of the plant as a whole. The same is true of the local action of a long daily light period in maintaining vegetative development and inhibiting flowering and fruiting.

## SUMMARY

In earlier investigations it was seen that when two coordinate branches of an individual plant of *Cosmos bipinnatus* are exposed to light for daily periods of different length each branch responds in characteristic manner to its particular light period more or less independently of the other branch. Continuing the investigations, experiments have been made with *Cosmos sulphureus*, a typical short-day plant, in which different portions of the primary stem were exposed to different daily periods of illumination, and, in some instances, to continuous darkness.

When the upper portion of the plant was exposed to the full length of day of summer while the lower portion received only 10 hours of light daily, the latter promptly flowered while the former remained vegetative. These localized responses might, it is supposed, continue for several months under favorable conditions.

Thus each portion of the axis has responded to its particular light period, in much the same manner as if it had been a separate plant.

Definite localization of the response was likewise obtained when the upper portion was subjected to a short daily period of illumination, and when the lower was exposed to a long one, and the former flowered readily while the latter continued to develop vegetatively.

When the central portion of the axis was exposed to a long day while both the lower and uppermost portions were subjected to the action of a short day, flowering was initiated in both the latter portions of the axis while the central portion continued to develop only vegetative shoots, further illustrating the possibility of sharply localizing the response to length of day.

On the other hand, when the central portion of the axis was exposed to a short day and the upper and lower portions to a long day, flower buds appeared in the former while both of the latter remained vegetative.

When the upper portion of the stem was excluded from light continuously for a period of 3 to 5 weeks while the lower portion received the light of a short day and consequently was forced into flowering, formation of flower buds was induced in the upper, darkened portion, and open blossoms appeared after the top had been returned to the

66850—25†——5

full length of day. When, however, the lower portion of the axis received the light of the long summer day and therefore was prevented from flowering, while the upper portion was maintained in continuous darkness, the latter formed no flower buds and resumed vegetative activity when returned to the light.

When the entire plant was excluded from the light for a period of 10 days and then returned to the action of the long day, flower buds did not appear.

These tests seem to indicate that, under the conditions, continuous darkness in itself does not definitely initiate flowering, but that, on the other hand, it does not necessarily inhibit formation of flower buds in response to the action of a short daily light period on another part of the plant.

# BEHAVIOR OF PHYTOPHAGA DESTRUCTOR SAY UNDER CONDITIONS IMPOSED BY EMERGENCE CAGES[1]

By WALTER H. LARRIMER

*Entomologist, Office of Cereal and Forage Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

## INTRODUCTION

The Hessian fly, *Phytophaga destructor* Say, has long been recognized in America as a wheat pest of primary importance, and even before its scientific description by Thomas Say in 1817 it had attracted considerable attention and was referred to under the popular name of Hessian fly. Naturally such an insect pest has been the subject of much published discussion as well as almost every conceivable type of investigation. Many excellent accounts of the life history, food habits, natural enemies, control, and other interesting phases in the life of this insect are readily available and therefore need not be repeated. Osborn (*7*)[2] has given a very comprehensive treatise on the Hessian fly in America, with a complete bibliography of all important papers up to 1898. For contributions appearing since that time full references will be found in the Bibliography of American Economic Entomology (*1*, *8*).

The determination of the time and extent of the emergence of adult flies from flaxseeds under varying conditions has always been an important factor in the study of this phase of the life history of the Hessian fly, and particularly in reference to various schemes for control. The present discussion deals with the design of a number of emergence cages, and the determination of the relative efficiency both as to approach to natural conditions and ability to recover emerged flies.

## EMERGENCE CAGES

Although many types of cages have been suggested from various sources, the selection of type, actual design of cages, and accomplishment of results may be considered as original. The six types of emergence cages selected were as follows:

TYPE A.—Cone-shaped, frame of sheet tin covered with 18-mesh pearl screen wire. Base circular, of diameter sufficient (40.6 inches) to make it inclose 1 square yard. Altitude 30 inches, and top terminating in a sheet-tin cone with 0.5-inch hole at the small end. Over this hole is fitted a small screen trap for catching flies that emerge from the cage through the hole in the top (fig. 1, A).

TYPE B.—Square, base inclosing 1 square yard. Height, 18 inches. Frame of 1 by 2 inch cypress, covered with 18-mesh pearl screen wire. Top is painted with thin tanglefoot and made to open upward to facilitate counting of flies caught on the tanglefoot (fig. 1, B).

[1] Received for publication Oct. 7, 1924; issued October, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 574.

Type C.—Square, base inclosing 1 square yard. Height, 18 inches. Frame of 1 by 2 inch cypress, covered with 18-mesh pearl screen wire. Top is made to open upward to facilitate counting of flies. The entire cage is lined with cheesecloth. Four single sheets of tanglefoot fly paper are fastened to the lower side of the top (fig. 1, C).

Type D.—This is a light-proof box of 0.5-inch pine, with base inclosing 1 square yard. In later experiments this type was made of a wood frame covered with tar paper. Two 29-millimeter glass vials were inserted through holes bored in the south side of the box (fig. 1, D).

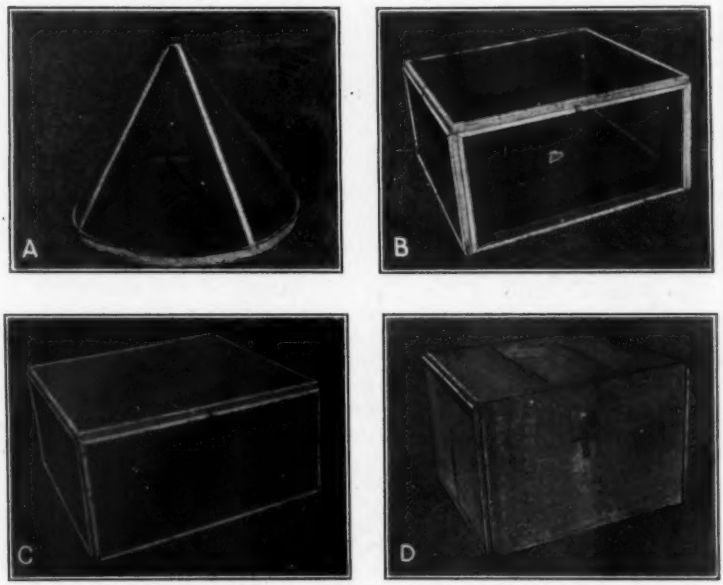



Fig. 1.—*A*, emergence cage for the Hessian fly, type A, having cone-shaped frame of sheet tin, covered with screen wire; B, emergence cage, type B, having square frame of cypress, covered with screen wire; *C*, emergence cage, type C, having square frame of cypress, covered with screen wire and lined with cheesecloth; *D*, dark emergence cage having frame of wood, made light-proof with tar-paper covering

Type E.—May be described as A-shaped, with square base inclosing 1 square yard. The two sloping sides are 1 yard square each and the ends A-shaped. Frame of 1 by 2 inch cypress, covered with 18-mesh pearl screen wire. Door, made by hinging one of the sloping sides at the top, is painted with thin tanglefoot, and the cage set so that this door faces south (fig. 2, A).

Type F.—This may be described as A-shaped with base square, inclosing 1 square yard; the two sloping sides 1 yard square each, and the ends A-shaped. Frame of 1 by 2 inch cypress, covered with 18-mesh pearl screen wire, with the exception of the door, which is made by having one of the sloping sides hinged at the top. This door is covered with 12-mesh pearl screen wire and painted with thin tanglefoot. The cage is set so that this door faces south (fig. 2, D).

FIRST TRIAL, NASHVILLE, ILL., APRIL 10 TO 30, 1917

The cages just described were arranged in a row extending east and west. Each covered 1 square yard of heavily infested winter wheat. There was reason to believe that each square yard was as nearly uniformly infested as was possible to obtain. However, there is some question as to whether an approximately equal number of flies might be expected to emerge in each cage. The cages were set in position April 9. During the 21 days of the experiment the total number of flies taken from each cage was as follows: A, 65; B, 63; C, 8; D, 134; E, 54; F, 61; total for all six cages, 385.

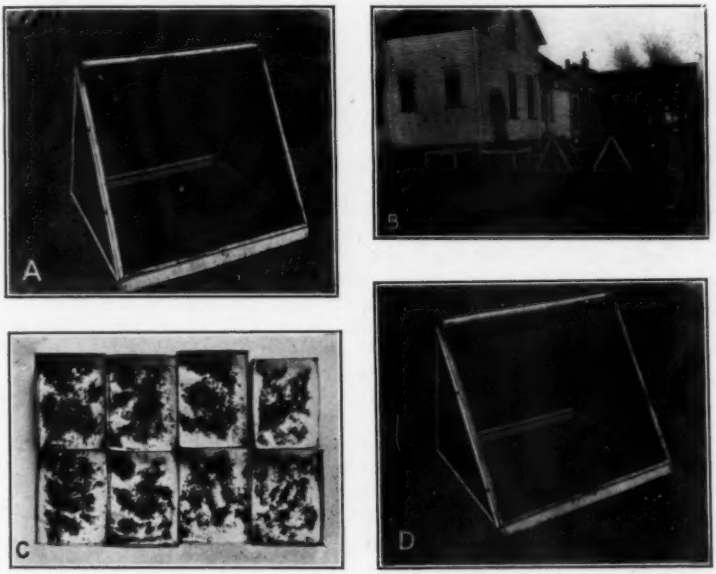



FIG. 2.—*A*, emergence cage for the Hessian fly, type E, having A-shaped frame of cypress, covered with 18-mesh screen wire; *B*, emergence cages in place for outdoor experiment; *C*, 12,000 "flaxseeds" or puparia used in indoor experiment; *D*, emergence cage, type F, having A-shaped frame of cypress covered with 18-mesh screen wire, except door, which is 12-mesh

The results obtained indicate that cage D was approximately twice as efficient in recovering flies as the best of the other cages. Cage C shows up very poorly, whereas the other cages are so close together in number of flies recovered that other considerations might be allowed to influence the selection of one of these cages for any particular purpose. A comparison of temperature, evaporation, and rainfall in each cage with that of natural conditions outside, or normal, was made. Temperature variations were determined by radiation thermometers placed close to the soil surface, and are shown in degrees plus or minus from the normal. Variations in evaporation were determined by compensating atmometers, and are shown in cubic centimeters plus or minus from the normal; the evaporation for each preceding 24 hours being recorded at 8 a. m. Variations in rainfall were determined by espe-

cially adapted standardized rain gauges and are shown in inches plus or minus from the normal, the amount for each preceding 24 hours being recorded at 5 p. m. Readings for each factor were made, and the accumulated variations are given in Table I.

TABLE I.—*Accumulated variation 19-day period, April 12 to 30, 1917*

| Factor | Cage A | Cage B | Cage C | Cage D | Cage E | Cage F | Time |
|---|---|---|---|---|---|---|---|
| Minimum temperature (°F.) | +34.5 | +32.0 | +22.5 | +51.5 | +26.0 | +31.5 | |
| Temperature (° F.) | +9.5 | −5.0 | −26.0 | −25.0 | −2.0 | −2.5 | 8 a. m. |
| | −21.5 | −33.0 | −103.0 | −141.5 | −39.5 | −17.5 | 12 m. |
| | −8.0 | −15.0 | −51.0 | −65.0 | −26.0 | −24.0 | 4 p. m. |
| Evaporation (c. c.) | −80.4 | −110.4 | −223.2 | −282.1 | −118.8 | −104.9 | 8 a. m. |
| Rainfall (inches) | −.49 | −.16 | −1.57 | −3.24 | −.53 | −.59 | 5 p. m. |

SECOND TRIAL, LA FAYETTE, IND., MARCH 15 TO 24, 1920

In order to make an extra trial, it was decided to run the cages indoors, thus forcing the adult flies to emerge by artificial heat. The cages were arranged in the laboratory under conditions as nearly uniform as possible.

The disadvantage pointed out in the first trial, namely, the existence of an uncertainty as to whether about the same number of flies emerged in each cage, was recognized, and an attempt made to eliminate it. Twelve thousand "flaxseeds" (fig. 2, C) were taken from wheat plants killed by the regular fall brood of the fly, and after being thoroughly mixed so that the entire lot would be uniform, they were divided into smaller lots, and 1,500 each were placed over moist soil in small rectangular tin boxes, and then exposed, one box in each cage. An additional 1,500 flaxseeds were placed in tin parasite boxes, and the adult flies allowed to emerge into glass vials. By counting these daily, and also those in the boxes, a suitable control was obtained on the total number which could reasonably be expected to emerge from each 1,500 flaxseeds in each of the cages.

Flies were counted and removed from the cages daily, as in the preceding trial, except that the record of males and females was kept separate, as follows:

Cage A, 205 females, 539 males, total 744; B, 127 females, 453 males, total 580; C, 21 females, 32 males, total 53; D, 307 females, 355 males, total 662; E, 195 females, 527 males, total 722; F, 136 females, 435 males, total 571; control, 512 females, 601 males, total 1,113.

No attempt is made to consider this trial as being conducted under any other than extremely unnatural conditions; however, it does give considerable information on the subject. It brings out the fact that although moisture and temperature may be the controlling factors in the emergence of adult flies, wind is certainly of some importance in the determination of cage efficiency. Indeed, the percentage of recovery is very remarkable in most cases, the exception being cage C, which will undoubtedly prove to be too inefficient to merit further consideration. Another remarkable point brought out in this experiment is the fact that from 1,500 flaxseeds used in the control, 1,113 adult flies were obtained, thus indicating that the very high rate of 74 per cent of the flies survived the winter, parasitism, and other

fatalities. Of the total number of flies emerging in the control, 601, or 54 per cent, were males, and 512, or 46 per cent, were females, in this case indicating a very fair degree of equality in the proportion of sexes. Another interesting point, although to be expected, is that of the recovery of the greater percentage of males in comparison with the females in each cage except the dark cage D. Excepting the cage C, there does not seem to be any choice indicated by the percentage of efficiency when the cages are run indoors.

A hygrothermograph was run in cage A, and temperature and humidity charts (Table II) were kept.

TABLE II.—*Temperature and humidity, La Fayette, Ind., March 15 to 24, 1920*

| Date | Temperature | | | Humidity | | |
|---|---|---|---|---|---|---|
| | Minimum | Maximum | Average[1] | Minimum | Maximum | Average[1] |
| | ° *F.* | ° *F.* | ° *F.* | *Per cent* | *Per cent* | *Per cent* |
| Mar. 15 | 65 | 75 | 70.5 | 38 | 49 | 43.8 |
| Mar. 16 | 60 | 72 | 65.8 | 35 | 40 | 38.4 |
| Mar. 17 | 58 | 70 | 64.4 | 34 | 36 | 34.7 |
| Mar. 18 | 58 | 68 | 64.2 | 36 | 39 | 37.5 |
| Mar. 19 | 62 | 68 | 64.6 | 37 | 44 | 39.7 |
| Mar. 20 | 58 | 74 | 68.1 | 35 | 38 | 36.2 |
| Mar. 21 | 62 | 77 | 71.4 | 33 | 36 | 34.2 |
| Mar. 22 | 65 | 77 | 71.1 | 33 | 37 | 35. ( |
| Mar. 23 | 68 | 76 | 73.5 | 31 | 37 | 34. 2 |
| Mar. 24 | 60 | 78 | 72.4 | 36 | 44 | 40.6 |

[1] The average is the arithmetical mean of readings at 2-hour intervals.

THIRD TRIAL, LA FAYETTE, IND., APRIL 22 TO MAY 12, 1920

The third trial was made in the same manner as the second, with the two main exceptions that the cages were placed under natural conditions outdoors and arranged in a row, as shown in Figure 2, B. Flaxseeds for this trial were collected and kept under as nearly natural conditions as possible until placed in the cages. One thousand flaxseeds were placed in each cage and protected from the sun's rays by small canopies of cheesecloth over each box. The results obtained are as follows: Cage A, 78 females, 191 males, total 269; B, 74 females, 88 males, total 162; C, 20 females, 12 males, total 32; D, 130 females, 233 males, total 363; E, 49 females, 100 males, total 149; F, 39 females, 55 males, total 94; control, 524 females, 240 males, total 764.

Cage D is again indicated to be the most efficient, and it is unfortunate indeed that this cage should impose conditions which are further from natural conditions than those of any of the variously devised cages of the experiments. Although in this trial, as proved by the control, the emergence was heavy, the number of flies recovered by the cages falls far below the indoor records. From 1,000 flaxseeds of the control 764 flies were obtained, a yield of 76 per cent. This comes very close to the indoor record, which was 74 per cent.

Attempts at further trials of these cages on May 11 and again on June 18, 1920, were made by placing flaxseeds in refrigeration to retard their development until after the natural emergence had occurred in the field. For some reason a sufficient emergence was

not obtained from these two lots of flaxseeds, and the scattering results will not be included.

Again, on September 15, 1920, another lot of flaxseeds was started in the cages, but owing to the freakish circumstance that adults emerged about 20 days later in the season than usual the number finally recovered by the cages was too small to give any data worth recording. In a faithful daily examination of the cages made throughout the period, it was discovered that ants were attacking and carrying away the flaxseeds; because of the unforeseen and extremely unusual delay in emergence, they were able to destroy quite a large number of them. This is a condition likely to occur whenever the cages are used, and it can not be considered other than a disadvantage.

## DISCUSSION

Of a total of 38,500 flaxseeds used in these experiments, records were made on 17,500, from which one may expect 13,125 flies to have emerged, and of these 6,278 were recovered by the various cages. An additional 385 were recovered by the cages in the first trial, making a total of 6,663 flies recovered and counted during the experiment.

Probably the best arrangement of the information obtained in the preceding experiments, and one by which a comparison can be made for selection of a cage for any special purpose, is that of a tabulation of the relative efficiency of the cages used. Table III shows, on a percentage basis, the conditions in each cage as compared to natural conditions—the natural temperature, evaporation, and rainfall being taken as 100 per cent. Efficiency in recovering flies is shown by the average per cent recovered from the number reasonably supposed to have emerged in each cage. The results from the indoor trial were not included in this rating, since conditions under which this particular experiment was run were unique.

TABLE III.—*Cage efficiency, on percentage basis*

| Cage | Temperature | | | | Evaporation | Rain | Flies recovered |
|---|---|---|---|---|---|---|---|
| | Minimum | 8 a. m. | 12 m. | 4 p. m. | 8 a. m. | 5 p. m. | |
| A | 95 | 99 | 99 | 99 | 76 | 85 | 35 |
| B | 96 | 99 | 97 | 90 | 66 | 95 | 21 |
| C | 97 | 97 | 92 | 96 | 33 | 58 | 4 |
| D | 94 | 97 | 89 | 95 | 15 | 00 | 48 |
| E | 97 | 99 | 97 | 98 | 64 | 84 | 20 |
| F | 96 | 99 | 99 | 98 | 68 | 82 | 12 |
| Control | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

It is found that cage A comes the nearest to being suitable for the determination of the date when flies are emerging in the field. For the actual recovery of flies, the dark cage D was the most efficient. For counting the flies, cages B, E, and F have their advantages, whereas cage C seems to be entirely impractical, as is shown in Table III.

## PRACTICAL APPLICATION

A discussion of the use that has already been made of this information on emergence cages in investigations that have been conducted subsequent to those of cage development may be appropriate. The dark cage D has been the one adopted in most cases, and many unpublished data on various treatments of stubble as a fly control have been compiled. Although this may be said to be the primary purpose of these cage experiments, it was also desirable to have a cage for use at "emergence stations" in comparison with migration screens and egg counts, as has been described by Gossard and Eastwood (*4*), Gossard and Parks (*5*), and later at "observation stations" as described by Drake, Fenton, and Butcher (*3*). The dark cage has given very satisfactory results in both cases, and when properly manipulated under the immediate direction of a trained entomologist, serves as a good indicator of Hessian-fly emergence.

It might be well to discuss briefly the results of experiments with emergence stations and the part played by emergence cages, the detailed report of which will be published later. Such stations have been in operation at La Fayette, Ind., and Centralia, Ill., during the fall emergence of the fly for the years 1919 to 1923 inclusive, that at the latter location being under the immediate observation of W. B. Cartwright, of the Bureau of Entomology, United States Department of Agriculture.

After this rather extensive use of emergence cages, migration screens, egg counts, and flaxseed examinations, dependence is now placed almost entirely on egg counts and flaxseed examination to give the emergence records which are most closely associated with infestation of wheat. The reasons for this are the advantages of simplicity, high degree of responsiveness, economy, and accuracy. Many midges closely resemble Hessian flies in cages and on migration screens, whereas there is very little chance of mistaking anything for a Hessian fly egg. The flaxseed examination to determine the status of pupation proved to be a very important contribution to the emergence-station activities.

The results obtained by this series of emergence stations have certainly demonstrated their value in the scientific study of this phase of the life history of the fly. Confidence in the ability thus to predict the safe time to sow wheat any one year has not been experienced to nearly the extent that has been the case elsewhere. Without flaxseed examination, a peak of emergence may be obtained, but there is nothing to indicate that there may not be another peak. Even with the status of pupation determined by flaxseed examination, actual emergence must occur before it can be heralded as such. By the time complete pupation and emergence have been determined, the sowing date will have been delayed five to seven days longer than necessary, a very vital matter to the farmer with some sizable acreage to sow. If regular sowing time arrives and passes and still a large proportion of the fly remains in the larval stage in the flaxseeds, just what policy is to be adopted? There can be no assurance that such larvae will remain in that stage until the following spring. They may pupate the very next few days after the safe sowing date has been announced, and thus emerge at exactly the right time to do the most damage to young wheat. Such cases have occurred too

66850—25†——6

frequently (*2*, *6*), and during the last five years in the East Central States have constituted the only really serious factor affecting the reliability of sowing dates determined by observations over a series of years.

Moreover, in years of moderate infestation, when the wave of infestation is on the up-grade, but because of lack of parasites considerable menace exists, it will be extremely difficult, if not entirely impracticable, to find enough flaxseeds for examination. In actual practice, even in years of heavy infestation, when parasitism has been heavy but not sufficiently so to remove the fly menace, considerable difficulty has been met in getting satisfactory records, and in some cases it was not at all practical to get them. Entirely successful cases of emergence have been obtained, but these almost without exception, have occurred in regular years when the emergence fitted in quite nicely with the established and set series of dates.

It would seem, therefore, that the practical application of the emergence cage to Hessian-fly investigations is confined principally to life history and control studies of other than a predictive nature.

## LITERATURE CITED

(1) American Association of Economic Entomologists.
1925. index to the literature of american economic entomology january 1, 1905, to december 31, 1924. 3 v. Melrose Highlands, Mass.

(2) Cartwright, W. B.
1923. delayed emergence of hessian fly for the fall of 1922. Jour. Econ. Ent. 16: 432–435.

(3) Drake, C. J., Fenton, F. A., and Butcher, F. D.
1923. hessian fly control in iowa. Iowa Agr. Exp. Sta. Circ. 86, 11 p., illus.

(4) Gossard, H. A., and Eastwood, G. R.
1916. county cooperation to control hessian fly. Ohio Agr. Exp. Sta. Mo. Bul. 1: 230–232.

(5) ——— and Parks, T. H.
1921. hessian fly prevention. Jour. Econ. Ent. 14: 53–60.

(6) Larrimer, W. H.
1922. an extreme case of delayed fall emergence of hessian fly. Ann. Ent. Soc. Amer. 15: 177–180, illus.

(7) Osborn, H.
1898. the hessian fly in the united states. U. S. Dept. Agr., Div. Ent. Bul. 16, n. s., 58 p., illus.

(8) United States Department of Agriculture, Bureau of Entomology.
1889–1905. bibliography of the more important contributions to american economic entomology. 8 pts. Washington, D. C.

# THE REACTION OF CERTAIN GRASSES TO CHINCH-BUG ATTACK [1]

By WM. P. HAYES, *Formerly Assistant Entomologist, Kansas Agricultural Experiment Station*, and C. O. JOHNSTON, *Assistant Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*

## INTRODUCTION

The bunch grasses of the prairies in the natural state are known to be the hibernating place of the chinch bug (*Blissus leucopterus* Say), which migrates from these grasses to cultivated members of the grass family (Poaceae), such as wheat, corn, and the sorghums. It is of special interest, therefore, to know what occurs when other grasses are attacked, both the effect on the grass, and, either because of slight injury or recovery, the extent to which it might serve as a hibernating medium. Chinch bugs have been recorded as attacking a number of grasses, but there seems to be no extended discussion in the literature on the importance of the grass family, other than cultivated crops, as food for chinch bugs. Webster [2] and Horton and Satterthwait [3] list a number of wild or native grasses attacked by this species. The present paper, besides greatly extending the previous lists of known food plants, seeks to show the degree of injury to the host plants and their ability to recover from injury.

During the summer of 1924, an opportunity occurred to record the data assembled here. A special grass garden of the Department of Botany and Plant Pathology of the Kansas Agricultural Experiment Station, containing about 100 grasses, used primarily for studies on the overwintering of leaf-rust of wheat, was located at the station on land adjoining wheat plots of a winter-wheat nursery in which there appeared large numbers of chinch bugs. The wheat was cut about July 1, and the bugs migrated to the adjacent grass garden. By July 8, injury to the various grasses was manifested in such varying degrees that it was deemed advisable to record the differences. A few weeks later, the chinch bugs left the grasses and journeyed to neighboring corn and sorghum. Rainfall was abundant after August 1, and some plants began to show signs of recovery. By August 18 the differences were so marked that notes on the ability to recover were taken.

## CULTURE OF GRASSES

The grass garden has been maintained for a number of years and many of the rows consisted of large, robust plants at least two years of age. In maintaining this garden those grasses which survive

[1] Received for publication Dec. 8, 1924; issued October, 1925. Contribution No. 339, Department of Entomology, and No. 230, Department of Botany. This paper embodies some of the results obtained in the prosecution of Projects Nos. 76 and 92 of the Kansas Agricultural Experiment Station.

[2] WEBSTER, F. M. THE CHINCH BUG. U. S. Dept. Agr. Farmers' Bul. 657, p. 7, 1915.

[3] HORTON, J. R., and SATTERTHWAIT, A. F. THE CHINCH BUG AND ITS CONTROL. U. S. Dept. Agr. Farmers' Bul. 1223, p. 35, 1922.

the winter are retained for further study, while those perennials which winterkill and all annuals are replanted each spring. To insure satisfactory growth it has been found necessary to plant the seed in small flowerpots in the greenhouse about March 1. This gives the seedlings a good start. After danger of severe frost injury is past, which is usually about April 1, they are transplanted to rows in the garden. This method of culture gives most of the grasses an excellent early-season growth. There are usually a few instances in which this treatment produces either extremely early or very late maturity. Such cases are rare, however, and therefore of no great significance. Then the grasses are transplanted to the garden rows, the plants are knocked from the pots and divided into small tufts, care being exercised to avoid any injury to the roots. The tufts then are carefully set in shallow trenches, and the soil firmed around them. The plants are placed in rows 5 feet long and 2 feet apart. This usually gives a considerable number of plants for observation, and prevents serious competition between species in adjacent rows.

The grasses made an exceptionally good start in the spring of 1924. Most of the rows had excellent stands. Although only a few plants were obtained in some rows, these were always normal. The early spring conditions so greatly favored the growth of grasses that most species had made a large amount of leaf growth before the chinch-bug migration from the wheat to the grasses had begun.

## FACTORS CONSIDERED

### ANNUAL OR PERENNIAL

One of the first plant characters to be considered in a study of this nature is the longevity of the plant. A short-lived annual might reasonably be expected to make an earlier and more vigorous growth than longer-lived perennials. In making observations on the chinch-bug injury to the grasses, it was noted that perennial species consistently exhibited much more resistance to attack than annuals. This may be due in part to the fact that most of the perennials had harsh leathery tissues, while most annuals are characterized by tender tissues which often are thin and sometimes rather flaccid. The chinch bugs attacked the annuals first and within a few days killed most of them.

It is realized that some plants are listed as annuals which, under other conditions, may be perennial, and vice versa. In some years at Manhattan certain species may survive the winter, while in others the same species may be completely killed. The habit given in this paper refers, therefore, to the behavior of the species under Kansas conditions.

### NATIVE OR INTRODUCED

In taking notes upon the amount of chinch-bug injury in the grass garden, it was observed immediately that species indigenous to Kansas exhibited striking resistance. It was further noted that the species native and common to that part of the State in which chinch-bug injury to cultivated crops most commonly occurs showed the greatest tolerance to attack. Furthermore, those same grasses comprise by far the greater percentage of the plants found in pastures, meadows, sod roadsides, and undisturbed fence rows. These are

the plants in which the chinch bugs overwinter in their normal habitat. On the other hand, grasses not native to Kansas were usually found to be severely injured, although in many cases they possess plant characters which were commonly found associated with resistance in indigenous species. Different species of the same genus, which vary primarily only in their natural range, also showed marked differences in their reaction to chinch bugs. Therefore, in this study the terms "native or introduced" are not used in the usual sense, but refer only to whether the species is native to Kansas or not.

### CHARACTER OF TISSUES

A wide difference was noted in the character of the plant tissues of different species. In general, however, the plants could be grouped into three classes: (1) Those having noticeably harsh, tough, or leathery tissues; (2) those with soft or tender tissues; and (3) those with tissues intermediate between the two extremes. In Table I these characters are indicated in the column headed "character of tissues" as harsh, tender, and intermediate. Grasses with harsh tissues are harsh to the touch, are glabrous or only slightly pubescent, and usually are rigidly erect as to leaf and culm characters. Harshness in the grasses has been ascribed by some writers to a high percentage of silicon in the vegetative parts. Tender-tissued plants are pubescent, as a rule, though not always so. The leaves are often thin and soft to the touch and are commonly drooping, thus giving a somewhat flaccid appearance to the plants. This is particularly true of the annual species of Bromus. The third group possesses tissues intermediate in character. Plants of this group may be pubescent or glabrous, but the tissues are leathery rather than harsh or tender.

It is interesting to note that such species as *Sporobolus cryptandrus*, *S. asper*, *Sorghastrum nutans*, *Eragrostis trichodes*, *Elymus virginicus*, *E. canadensis*, *Triodia flava*, *Bouteloua oligostachya*, *B. curtipendula*, and *Andropogon scoparius*, which exhibited the greatest resistance to chinch-bug attack, are all perennial native species with harsh tissues. These are also the commonest roadside, pasture, and prairie grasses in the vicinity of Manhattan.

### TIME OF MATURITY

The time of maturity refers to the period during which each species has been observed to flower and mature seed at Manhattan. In a number of cases, however, where new species were being grown for the first time and no first-hand information on this point was available, it was obtained from published floras. A few of the species matured before the chinch-bug attack, but the majority would not have matured until afterward. Plants of related species in various stages of growth were available for observation. In a few cases, plants of the same species of various ages were available. *Hystrix hystrix* was a case in point. The plants in row 35 were large robust plants, two years old, while those in row 73 were young plants grown from the seed in the spring of 1924. In this instance the older plants were attacked and killed by the bugs as readily as the young plants. In fact, no relation was noted between the stage of maturity of the plants and their reaction to chinch bugs. It is, however,

important to have information on the maturity of species from the standpoint of overwintering and early spring feeding of the bugs. Those species which mature extremely early usually are blown away before fall and therefore would afford very little winter protection, while the later-maturing species retain their dead stems and leaves throughout the winter, making excellent chinch-bug harbors. Most of the common native perennial grasses found in the vicinity of Manhattan are of that nature.

### TYPE OF PLANT GROWTH

The characteristic types of growth exhibited by the plants under culture are noted as turf-forming, dispersed, tufted, and bunch. The terms "turf-forming" and "bunch" are self explanatory. In those species of dispersed habit, the plants usually are found singly or scattered over a considerable area. Such plants usually have only a few culms and do not form definite tufts or clumps, although there may be a large number of plants in a small area. The plants occurring in tufts are usually low-growing species, such as species of Bouteloua; while bunch types are usually large and coarse-stemmed, such as species of Andropogon, *Sorghastrum nutans*, and *Triodia flava*.

### HABITAT

Information concerning the habitats of the various species was obtained from a number of sources. That given for the species indigenous to Kansas was taken from the junior author's notes and experience with those species. For the exotic species, knowledge of the habitat was obtained from various publications on the flora of the United States and Europe. Wherever possible the kind of place in which the species occurs most commonly is referred to, in this paper, as its habitat. It is of considerable interest to note that those grasses which were the most resistant to chinch-bug attack were those which occur most commonly as roadside or fence-row grasses, and comprise most of the pasture and prairie grasses indigenous to Kansas. Two roadside and fence-row conditions obtain in Kansas, one in which the original sod has not been disturbed, and in which the native perennial prairie grasses predominate, and another in which the soil has been disturbed and annual species such as *Bromus tectorum*, *B. arvensis*, *Hordeum pusillium*, *H. jubatum*, and annual species of Fragrostis predominate.

There are a number of species which are listed as occurring in cultivated fields. Such grasses as *Bromus tectorum*, *B. arvensis*, *Hordeum jubatum*, and species of Lolium often appear in wheat fields and sometimes become serious weeds. Within the last three years *Aegilops cylindrica* (*Triticum cylindricum*) has appeared as a noxious weed in wheat fields and disturbed roadsides and fence-rows in central Kansas. This species is of interest from the standpoint of chinch bugs, since it is a winter annual like wheat, often occurs as a volunteer weed in the late summer, and is so closely related to wheat as to hydridize with it.

### CHINCH-BUG INJURY

Within a few days after the adjacent wheat was harvested most of the rows of grasses were literally swarming with chinch bugs in all instars of nymphal development, as well as thousands of adult

insects. Many of the different grasses soon were killed or severely injured, while others showed varying degrees of resistance. Notes regarding these differences were taken on July 8. Since such data, at best, can be only relative, it was thought advisable to list the degree of injury in as few categories as possible. Accordingly the following degrees of injury were considered. If a row of grass was entirely dead it was noted as *killed;* more than half dead was considered *severe;* if only half dead it was regarded as *moderate;* and when only a few plants were injured it was listed as *slight.*

### PERCENTAGE OF RECOVERY

The observations on the ability of the various species of grasses to recover from injury were made on August 18. Very favorable growing weather had prevailed between this date and the time when the insects attacked the plants. By this date, also, most of the chinch bugs had become mature and had flown to neighboring corn and sorghum fields.

In estimating the percentage of recovery, rows in which no live plants were present were listed as zero, meaning no recovery; those in which only one or two plants were alive were marked 1 per cent, and so on, until those rows in which there seemed to be complete recovery were noted as 100 per cent. Recovery data for the few rows in which there was no injury are not given in Table I.

### PRESENTATION OF DATA

To the data on the amount of injury and the degree of recovery have been added the morphological and ecological features of the various grasses discussed in the previous paragraphs and these have been summarized in Table I.

## DISCUSSION

From Table I it seems strikingly apparent that the character of the vegetation and the circumstance of whether the species of grass is native or introduced, have considerable to do with the ability of the plant to withstand chinch-bug attacks. Those grasses having harsh tissues seem, in most cases, to be especially resistant, if they are native grasses. For example, such a plant as *Andropogon scoparius* (row 3) has harsh tissues and is a native perennial. It is well known that it affords the chinch bug protection during the winter, and, in the case noted, no injury was apparent, although the plants were literally covered with chinch bugs. In the majority of instances of those grasses termed "harsh" in which chinch-bug injury was severe and recovery poor, the species were introduced ones.

It will be observed further that those grasses listed as "intermediate" in regard to their vegetative characters showed varying degrees of resistance. Some, such as *Festuca ovina* (row 99), showed only slight injury, and with marked ability to recover. Others, like *Bromus secalinus* (row 57), showed only moderate injury and mediocre ability to recover. Still others were severely damaged, e. g., *Elymus glaucus* (row 13), and were unable to recover.

TABLE I.—*The reaction to chinch-bug attack of certain grasses in the grass garden, Kansas Agricultural Experiment Station, Manhattan, Kans.*

| Row | Scientific name | Common name | Annual or perennial | Native or introduced | Character of tissues | Time of maturity | Type of growth | Habitat | Chinch-bug injury | Per cent of recovery |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Bromus patulus | Spreading brome grass | A | I | Tender | July–Aug | Tufted | Waste places | Killed | 0 |
| 2 | Anthoxanthum odoratum | Sweet vernal grass | P | I | Harsh | June–July | do | Meadows | Slight | 70 |
| 3 | Andropogon scoparius | Little bluestem | P | N | do | Aug.–Sept | Bunch | Prairies and sod roadsides. | None | |
| 4 | Andropogon laguroides | | P | N | do | July–Sept | do | do | Slight | 100 |
| 5 | Bromus sp. (octoenensis?) | | A | I | Tender | May–July | Tufted | Waste places | Killed | 0 |
| 6 | Bromus arvensis | False cheat | A | N | do | do | do | Disturbed places, roadsides. | Moderate | 1 |
| 7 | Bromus lanuginosus | | A | I | do | June–July | do | Waste places | Severe | 0 |
| 8 | Hordeum bulbosum | | A | I | Harsh | May–June | Dispersed | do | do | 10 |
| 9 | Bromus arvensis | False cheat | A | N | Tender | May–July | Tufted | Disturbed places, roadsides. | Moderate | 0 |
| 10 | Bromus erectus | Upright brome grass | A | I | Harsh | July–Aug | Dispersed | Waste places | Slight | 100 |
| 11 | Agropyron lanceolatum | | P | I | do | July–Sept | do | Sod land roadsides | Severe | 1 |
| 12 | Bromus laevipes | | A | I | Tender | June–July | Tufted | Waste places | do | 0 |
| 13 | Elymus glaucus | Smooth wild rye | P | I | Intermediate | June–Aug | Bunch | do | do | 0 |
| 14 | Bromus pumpellianus | | P | I | Harsh | do | do | do | Slight | 100 |
| 15 | Hordeum maritimum | Seaside barley | A | I | do | June–July | Tufted | Sand shores, waste places. | (a) | |
| 16 | Elymus caput-medusae | | A | I | Intermediate | July–Sept | Bunch | Waste places | Severe | 20 |
| 17 | Lolium subulatum | | A | I | do | May–June | Dispersed | Meadows | do | 0 |
| 18 | Bromus breviaristatus | Short-awned cheat | A or P | I | Harsh | July–Aug | do | Waste places | Moderate | 3 |
| 19 | Bromus sitchensis | | P | I | do | July–Sept | Bunch | | Slight | 100 |
| 20 | Elymus striatus | Slender wild rye | P | N | do | June–July | do | Shady places | Severe | 1 |
| 21 | Elymus condensatus | | P | I | do | July–Aug | do | Waste places | Moderate | 40 |
| 22 | Bromus pratensis | | A | I | Tender | do | Tufted | | do | 20 |
| 23 | Bromus rigidus | Rigid brome grass | A | I | do | do | do | Waste places | Killed | 0 |
| 24 | Paspalum floridanum | Florida paspalum | P | I | Harsh | Aug.–Sept | Bunch | do | Slight | 100 |
| 25 | Lolium temulentum | Darnel | A | I | do | May–June | Dispersed | Cultivated fields waste places. | (a) | |
| 26 | Phalaris minor | Small canary grass | A | I | Tender | June–Aug | do | do | Killed | 0 |
| 27 | Sporobolus cryptandrus | Sand drop seed | P | N | Harsh | Aug.–Sept | Tufted | Sod-roadsides | Slight | 95 |
| 28 | Sporobolus airoides | Hair-grass drop seed | P | N | do | do | Bunch | Prairie, sod roadsides | Moderate | 100 |
| 29 | Sporobolus asper | Long leaved rush grass | P | N | do | do | do | do | None | |
| 30 | Sorghastrum nutans | Indian grass | P | N | do | do | do | do | do | |
| 31 | Hordeum caespitosum | | A | I | Tender | May–June | Dispersed | Waste places | (a) | |
| 32 | Bromus tectorum nudus | | A | I | do | June–July | do | Disturbed places | Killed | 0 |
| 33 | Agropyron violaceum | | P | I | Harsh | July–Aug | Turf | Roadsides | Severe | 0 |
| 35 | Hystrix hystrix | Bottle brush grass | P | I | do | June–July | Bunch | Sod places | Killed | 0 |
| 36 | Oryzopsis miliacea | Mountain rice | A | I | do | July–Sept | Dispersed | High altitudes | (a) | |
| 37 | Triodia flava | Purple top | P | N | do | Aug.–Sept | Bunch | Meadows, roadsides | Slight | 100 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 38 | Triodia albescens | | P | [c] I | do | June-Aug | do | Prairies | do | | 100 |
| 39 | Phalaris arundinacea | Reed canary grass | P | N | Intermediate | July-Aug | Dispersed | Moist, waste places | Moderate | | 100 |
| 40 | Bromus polyanthus | | A or P | I | Tender | do | Tufted | | do | | 95 |
| 41 | Bromus maximus (B. rigidus). | Great brome grass | A | I | do | do | do | | Killed | | 0 |
| 42 | Hordeum murinum | Wall barley | A | I | Intermediate | June-July | Dispersed | Waste places | do | | 0 |
| [c] 44 | Festuca rubra | Red fescue | P | I | Harsh | May-June | Turf | Cultivated | Slight | | 100 |
| 45 | Arrhenatherum elatius | Tall oats grass | P | I | do | June-Aug | Bunch | do | Severe | | 90 |
| 46 | Eragrostis trichodes | Blow-out grass | P | N | do | July-Sept | do | Sandy areas | Slight | | 100 |
| 47 | Eragrostis pilosa | Small tufted love grass | A | N | Intermediate | do | Tufted | Waste places | [b] | | |
| 48 | Eragrostis lugens | | P | I | do | Aug.-Sept | Bunch | Prairies | [c] | | |
| 49 | Hordeum gussoneanum | | A | I | Tender | June-July | Dispersed | Waste places | Killed | | 0 |
| 50 | Bromus sterilis | Barren brome grass | A | I | do | do | do | do | do | | 0 |
| 51 | Bromus villosus (B. rigidus) | | A | I | do | do | do | do | do | | 0 |
| 52 | Bromus rubens | | A | I | do | do | do | do | do | | 0 |
| 53 | Lolium temulentum [f] | Darnel | A | I | do | do | do | Cultivated fields | [b] | | |
| 54 | Hordeum jubatum | Squirrel-tail grass | A | N | Harsh | do | do | Fence rows | Killed | | 0 |
| [c] 56 | Bromus unioloides | Rescue grass | A | I | Intermediate | May-July | do | Meadows, pastures | do | | 0 |
| 57 | Bromus secalinus | Cheat | A | N | do | do | do | Fence rows, roadsides | Moderate | | 20 |
| 58 | Bromus tectorum | Downy brome grass | A | N | Tender | May-June | do | Waste places | Severe | | 0 |
| 59 | Bromus inermis | Hungarian brome grass | P | N | Harsh | June-Aug | Turf | Roadsides | Slight | | 100 |
| 60 | Hordeum nodosum | Meadow barley | A | I | Intermediate | May-July | Dispersed | Waste places | [b] | | |
| 61 | Agropyron pungens | Coast wheat grass | P | I | Harsh | July-Aug | do | Sandy places | Moderate | | 2 |
| 62 | Leptochloa fascicularis | Spike grass | A | N | do | May-Aug | do | Waste places | [b] | | |
| 63 | Elymus virginicus | Virginia wild rye | P | N | do | July-Aug | Tufted | Moist places, fence rows | Moderate | | 50 |
| 64 | Bromus kalmii | Kalm's cheat | A | I | Tender | do | Dispersed | Woods and thickets | Killed | | 0 |
| 65 | Dactylis glomerata | Orchard grass | P | N | do | June-July | Bunch | Cultivated | Moderate | | 60 |
| 66 | Agropyron caninum | Awned wheat grass | A | I | do | July-Aug | Dispersed | | Killed | | 0 |
| [c] 68 | Festuca elatior | Meadow fescue | P | I | do | do | Tufted | Cultivated | Moderate | | 95 |
| 69 | Stipa viridula | Feather-bunch grass | P | I | do | do | Bunch | Prairies | Severe | | 10 |
| 70 | Bouteloua gracilis | Blue grama grass | P | I | do | do | Tufted | do | Slight | | 100 |
| 71 | Elymus canadensis | Canada lyme grass | P | N | do | do | Bunch | Roadsides | Severe | | 95 |
| 72 | Phalaris caroliniana | Carolina canary grass | A or P | I | Intermediate | June-Aug | Dispersed | Waste places, moist soils. | do | | 1 |
| 73 | Hystrix patula [e] (H. hystrix) | Bottle-brush grass | P | I | Harsh | do | Bunch | Sod places | Killed | | 0 |
| 74 | Lolium sp. (argentium) | | A | I | Intermediate | May-July | Dispersed | | [b] | | |
| 75 | Festuca feras | | P | I | Harsh | June-July | Tufted | | Moderate | | [c] |
| 76 | Agrostis verticillata | Small bent grass | A | I | Tender | May-July | do | Waste places | Severe | | 40 |
| 77 | Festuca bromoides | Barren fescue | A | I | do | June-July | Dispersed | do | Killed | | 0 |
| 78 | Paspalum setaceum | Straw-colored paspalum | A | N | do | Aug.-Sept | Tufted | Waste, sandy places | Moderate | | 95 |
| 79 | Chloris verticillata | Prairie chloris | P | N | Harsh | July-Sept | do | Prairie roadsides | Slight | | 100 |
| 80 | Deschampsia flexuosa | Wavy hair grass | P | I | do | June-Aug | Turf | Waste places | do | | 100 |
| 81 | Bouteloua curtipendula | Side-oats grama | P | N | do | Aug.-Sept | Bunch | Prairies, sod roadsides | do | | 100 |
| 82 | Agrostis perennans | Upland bent grass | P | I | do | Aug.-Oct | Tufted | Dry sod | Moderate | | 10 |
| 83 | Alopecurus geniculatus | Marsh foxtail | A | N | Tender | May-June | Dispersed | Moist, waste places | [b] | | |

[a] Died from other causes before bug attack.
[b] Matured before bug attack.
[c] These occur rarely in southwestern Kansas; have been introduced into the Manhattan locality.
[d] In rows Nos. 34, 44, 55, 67, 91, 92, and 95, no stand was obtained.
[e] *Hystrix hystrix=Hystrix patula.* Row 35 received from Indiana (2-year-old). Row 73 received from Idaho (seedlings).
[f] Awned type; typical *L. temulentum* is awnless.

TABLE I.—*The reaction to chinch-bug attack of certain grasses in the grass garden, Kansas Agricultural Experiment Station, Manhattan, Kans.*—Continued

| Row | Scientific name | Common name | Annual or perennial | Native or introduced | Character of tissues | Time of maturity | Type of growth | Habitat | Chinch-bug injury | Per cent of recovery |
|---|---|---|---|---|---|---|---|---|---|---|
| 84 | Festuca tenufolia | | A or P | I | Intermediate | | Tufted | | Severe | 4 |
| 85 | Bouteloua oligostachya (B. gracilis). | Grama grass | P | N | Harsh | July–Sept | do | Prairies, sod roadsides | Slight | 100 |
| 86 | Festuca myuros | Rats-tail fescue | P | I | do | June–July | do | Waste places | Killed | 0 |
| 87 | Schedonnardus paniculatus | Texas windmill grass | P | N | do | do | do | Prairies, sod roadsides | Severe | 100 |
| 88 | Sphenopholus obtusata | | A | N | Tender | May–June | Dispersed | Meadows | Slight | 10 |
| 89 | Eragostis lugens | | P | I | Intermediate | July–Aug | Bunch | Prairies | Severe | 15 |
| 90 | Eragostis secundiflora | Clustered love grass | A | N | do | Aug.–Sept | Dispersed | Waste places, dry soil | Killed | 0 |
| *93 | Bouteloua oligostachya (B. gracilis). | Grama grass | P | N | Harsh | July–Aug | Tufted | Prairies and sod roadsides. | Slight | 100 |
| 94 | Sitanion hystrix | | P | I | do | do | do | Prairies | Killed | 0 |
| *96 | Anthoxanthum odoratum | Sweet vernal grass | P | I | do | June–July | do | Meadows | Moderate | 95 |
| 97 | Bromus hordeaceus | | A | I | Tender | July–Aug | Dispersed | Waste places | Killed | 0 |
| 98 | Pennisetum japonicum | | P | I | Harsh | Aug.–Sept | Bunch | Ornamental | Moderate | 10 |
| 99 | Festuca ovina | Sheep fescue | P | I | Intermediate | June–July | Turf | Cultivated | Slight | 100 |
| 100 | Aegilops cylindrica (Triticum cylindricum). | | W A[b] | N | Tender | do | Dispersed | Cultivated, fields | Killed | 0 |

* These occur rarely in southwestern Kansas; have been introduced into the Manhattan locality.

[b] Winter annual.

Among the plants with tender tissues, there was only one species, *Sphenopholus obtusata* (row 88), which showed but slight damage. A few species, for example *Bromus arvensis* (row 6), showed only moderate injury. The majority of tender grasses were either severely injured or completely killed.

One instance is noted, in the case of *Hystrix hystrix* (row 35), in which the age of the grass seemed to make no marked difference in the ability of the plant to withstand injury. Row 35 was a 2-year old stand of *Hystrix hystrix* seed of which originally came from Indiana. Row 73 was composed of seedling plants of the same species, seed of which was received from Idaho under the name of *Hystrix patula* and so listed in the plots. Resistance was no more noticeable in the 2-year-old strain than in the 1-year-old strain, for both were killed.

No correlation is apparent between habit of growth (that is, whether the plants are tufted or bunched) and the degree of injury and recovery. Bunch types were alike slightly injured or killed, and the same can be said of the turf, tufted, and dispersed types. The date of maturity of the various species has been listed herein because it was noted that several species had matured and produced seed before the migration of the chinch bugs from the wheat to the grasses. This fact may have an important bearing in badly-infested areas where the production of an early seed crop is desired.

No correlation is seen between habitat and degree of injury, although it is important to note that some grasses which occur as pests in cultivated fields, such as *Aegilops cylindrica* (row 100), may be killed by the bugs. Others which occur in plowed fields, such as *Lolium temulentum* (row 53), may mature and produce seed before the insect attack begins; while grasses like *Festuca elatior* (row 68), also found in cultivated areas, may show only moderate injury and marked recuperative powers.

## SUMMARY

During the season of 1924, the grasses in the grass garden of the Kansas Agricultural Experiment Station were attacked by large numbers of chinch bugs which migrated from adjacent wheat fields after harvest. The different species showed different degrees of resistance to injury, and later some of them exhibited marked ability to recover from the attack. It was apparent that native perennial species with harsh tissues were able to survive chinch-bug injury and showed the most marked ability to recover. These grasses comprise about 80 per cent of the native prairie grasses of Kansas.

Besides the data regarding the resistance and recovery of the grasses, a large number of species of grass not heretofore recorded as host plants of the chinch bug are listed.

# DIFFERENCE IN INTERNODE LENGTHS BETWEEN, AND EFFECT OF VARIATIONS IN LIGHT DURATION UPON, SEEDLINGS OF ANNUAL AND BIENNIAL WHITE SWEET CLOVER[1]

By A. J. PIETERS

*Agronomist, Clover Investigations, Bureau of Plant Industry, United States Department of Agriculture*

## HISTORICAL DATA

The effect of environment on the growth of plants has probably been studied more extensively by Klebs than by any other worker. He studied not only the effect of various salts, of temperature and moisture, but in his later papers touched on the effect of illumination for different periods on the blossoming of Sempervivum.[2] In this he showed that the blossoming of *Sempervivum albidum* and *S. funkii* could be affected by varying the hours of illumination to which rosettes were subjected while flower buds were forming. Later Garner and Allard [3] studied the effect of varying periods of illumination on a number of plants and established the fact that in many cases at least the blossoming of a species depends on the hours of illumination.[4]

In a second paper,[5] Garner and Allard touch on the apogeotropic response to photoperiodicity. They found that increasing the length of day increased the total stem length of *Amaranthus hispidus* and other plants, but that the reverse was true of sorghum and certain small grains. They do not state whether this lengthening of the axis was in the form of increase in the number of internodes or increase in the length of each internode. In *Melilotus alba*, the increased length was due wholly to an increase in the length of each internode.

The experiments just referred to showed, as did those of Oakley and Westover, that the length of the period of illumination may be expected to influence not only the blossoming but also the vegetative growth of the plant. Under a short day the internodes were shortened, while under a long day they were very greatly elongated. The degree to which the seedlings were affected varied somewhat with the variety. Doubtless other plants will be found to react in a similar manner.

## EXPERIMENTAL DATA

In August, 1921, seeds of annual and of biennial white sweet clover were planted in parallel rows at the Arlington Experiment Farm, Rosslyn, Va. On October 4, 1921, it was noted that the annual plants were taller than those of the biennial, though the number of

[1] Received for publication October 16, 1924; issued October, 1925.

[2] KLEBS, G. UEBER DIE BLÜTENBILDUNG VON SEMPERVIVUM. Flora (N. F. 11/12) 111/112: 128–151, illus. 1918.

[3] GARNER, W. W., and ALLARD, H. A. EFFECT OF THE RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. Jour. Agr. Research 18: 553–606, illus. 1920.

[4] As Garner and Allard have already adequately reviewed the literature in their two papers, no attempt to do so is made in this paper.

[5] GARNER, W. W., and ALLARD, H. A. FURTHER STUDIES IN PHOTOPERIODISM, THE RESPONSE OF THE PLANT TO RELATIVE LENGTH OF DAY AND NIGHT. Jour. Agr. Research 23: 871–920, illus. 1923.

leaves on both was about the same. The difference between them was evidently due to the greater internode length of the former (pl. 1). It was at once suggested that this difference might be utilized as a means of identifying the seed of the annual then coming into prominence and the seed of which is indistinguishable from that of the biennial.

On October 11, when 60 days old, these seedlings were taken up and measured from: (1) Cotyledons to tip of the longest leaf, and (2) cotyledons to growing point of the stem. Naturally, the exact location of the growing point could not be ascertained without elaborate dissection in each case. However, by careful observation, its position could be determined very closely, and the writer is confident that the error will not exceed $\pm 0.5$ mm.

In the seedlings of Melilotus, as in Trifolium, Medicago, and many other legumes, the first leaf is unifoliolate (pl. 1, *a*) and all subsequent leaves trifoliolate. Counting the unifoliolate leaf as one, the number of expanded or nearly expanded leaves is equal to the number of internodes above the cotyledons, though the upper internode may not yet have reached full length. By noting the number of expanded or nearly expanded trifoliolate leaves, a means is afforded for comparing seedlings of equal development.

In the work here reported, all seedlings taken up on a given date were separated into groups, each group containing those plants with the same number of expanded, or nearly expanded, trifoliolate leaves. The last internode lengthens as the upper leaf expands, and a part of the irregularities in the measurements recorded in Table I is due to unequal maturity of the upper internodes. The cotyledons [6] remain attached for many days or weeks and afford a convenient and fixed joint from which to measure (pl 1, *b*). The measurement from cotyledons to tip of longest leaf was found to be unsatisfactory, because of great variations in petiole lengths, and this measurement was abandoned. In measurements made subsequent to those recorded in Table I the length from cotyledons to first (unifoliolate) leaf, and the length from cotyledons to growing point were made. In all, 4,610 seedlings were measured, involving more than 9,000 measurements.[7]

TABLE I.—*Average stem and internode lengths in millimeters of seedlings measured October 11, 1921, 60 days after date of seeding*

| | Annual | | | Biennial | | |
|---|---|---|---|---|---|---|
| | 3-leaved [a] | 4-leaved | 5-leaved | 3-leaved | 4-leaved | 5-leaved |
| Average stem length | 22.4 | 35.8 | 45.55 | 7 | 10.8 | 11.1 |
| Average internode length | 5.6 | 5.16 | 7.6 | 1.75 | 2.16 | 1.85 |

[a] The number of internodes was one more than the number of trifoliolate leaves.

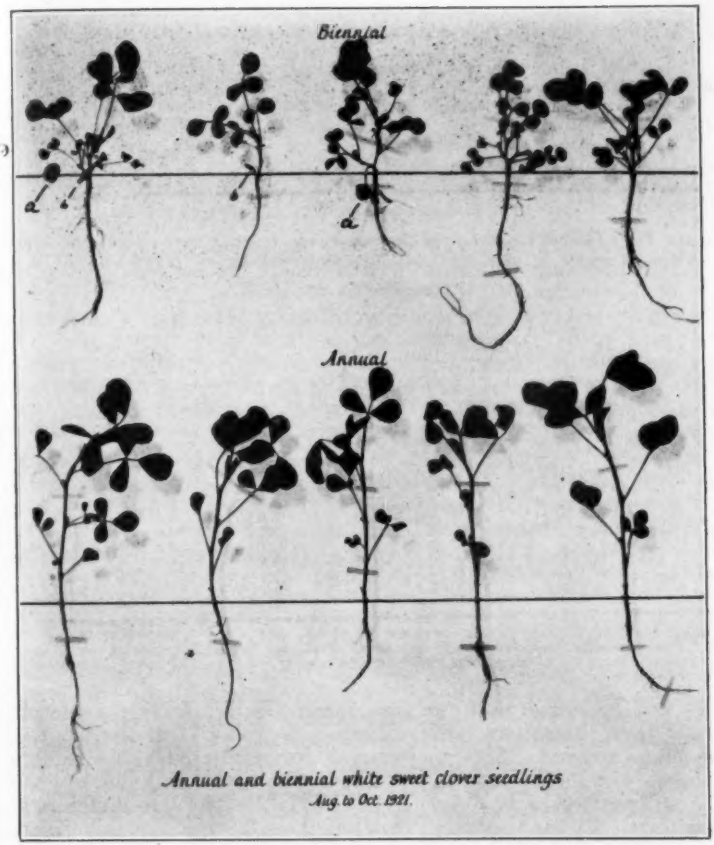
It will be seen from Table I that the length of the internodes in the annual was two, three, or more times as great as in the biennial (pl. 1).

These facts were reported at a meeting of the Association of Official Seed Analysts of North America, held in July, 1923.[8]

[6] In all plates showing mounted specimens, the plants have been so mounted that the cotyledons, or the point where they were attached, are on the heavy black line.

[7] The writer's thanks are due to L. W. Kephart for assistance in making these measurements.

[8] ASSOCIATION OF OFFICIAL SEED ANALYSTS. PROCEEDINGS OF THE FOURTEENTH AND FIFTEENTH ANNUAL MEETINGS. 148 p. 1923




Showing difference in stem and root development between biennial and annual white sweet clover grown outdoors August 10 to October 4, 1921

As a result of this first observation seeds of annual and biennial white sweet clover were sown in the greenhouse on December 24, 1921, and the flats subjected to various periods of illumination: (1) Normal day for that time of year, about 9½ hours;[9] (2) short day, from 9 a. m. to 4 p. m.; (3) long day, from daylight to dark, with illumination, from twilight until 11 p. m., by three 200-Watt electric lamps hung about 2 feet above the flats. The temperature of the greenhouse was 50 to 60° F. The rise of temperature caused by the electric lights was inconsiderable, as was that in the dark chamber.[10] Under these conditions the following six lots of seed were tested:

2066. Grundy County biennial, an early dwarf form.
2140. Commercial biennial.
03464. Arctic biennial, an early dwarf form.
2143. Mixed annual.
12055. A late annual selection.
12060. An early annual selection.

The seedlings were measured 51 days after sowing. The measurements are given in Table II.

TABLE II.—*Average length of the first internode, average stem length, and average internode length of seedlings, 51 days from sowing on December 24, 1921*

[Measurements in millimeters]

| | | 3-leaved | | | | | | 4-leaved | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Biennial | | | Annual | | | Biennial | | | Annual | | |
| | | 2066 | 2140 | 03464 | 2143 | 12055 | 12060 | 2066 | 2140 | 03464 | 2143 | 12055 | 12060 |
| N.* | I′ | 8.5 | 12.3 | 11.6 | 14.4 | 19 | 16.9 | 10.9 | 15 | 13.9 | 14.3 | 19.6 | 17 |
| | S | 14.3 | 22.4 | 17 | 25 | 36 | 30 | 22 | 29.3 | 26 | 33.5 | 45.5 | 38.1 |
| | I | 1.9 | 3.4 | 1.8 | 3.5 | 5.7 | 4.4 | 2.8 | 3.6 | 3 | 4.8 | 6.5 | 5.4 |
| S. | I′ | 3 | 5 | 8.5 | 6.2 | 6 | 5.3 | 4.8 | 5.5 | 8.6 | 6.8 | 7.1 | 5.5 |
| | S | 4.45 | 7.35 | 18.3 | 10 | 10 | 8 | 7.6 | 9.9 | 18.8 | 12.5 | 11.8 | 8.3 |
| | I | .5 | .8 | 3.3 | 1.3 | 1.3 | .9 | .7 | 1.1 | 2.5 | 1.4 | 1.2 | .7 |
| L. | I′ | 13.3 | 18.8 | 23.6 | 21 | 23.7 | 20.4 | 15.4 | 18.3 | 28 | 23.2 | 23.6 | 22.2 |
| | S | 28 | 40 | 43.5 | 44.3 | 45 | 40.9 | 40.2 | 48.5 | 57 | 58.8 | 58 | 55.7 |
| | I | 4.9 | 7.1 | 6.6 | 7.4 | 7.1 | 6.6 | 6.2 | 7.5 | 7.2 | 8.9 | 6.1 | 8.4 |

*N=normal day. S=short day. L=long day. I′=length first internode; cotyledons to unifoliolate leaf. S=stem length; cotyledons to growing point. I=average length all internodes above the first. This figure is found by dividing stem length from unifoliolate leaf to growing point by the number of trifoliolate leaves.

It will be noted that the first internode is always considerably longer than the others. For example, in Table II, if the length of the first internode of 2066, biennial, for normal day, is deducted from the total stem length, the length above the unifoliolate leaf is 5.75 millimeters, or an average of slightly less than 2 millimeters per internode. In 2143, annual, this length is 3.5 millimeters. In the three and four leaved seedlings, the length of the first internode, under normal day, equals or exceeds that of the entire stem above the unifoliolate leaf, but in older seedlings this relation changes. See Table III, where in the normal day columns for the seven-leaved seedlings, the average length of each internode above the unifoliolate leaf is but little less than that of the first internode.

[9] Data from GARNER, W. W., BACON, C. W., and ALLARD, H. A. PHOTOPERIODISM IN RELATION TO HYDROGEN-ION CONCENTRATION OF THE CELL SAP AND THE CARBOHYDRATE CONTENT OF THE PLANT. Jour. Agr. Research 27: 119–156, illus. 1924.

[10] OAKLEY, R. A., and WESTOVER, H. L. EFFECT OF THE LENGTH OF DAY ON SEEDLINGS OF ALFALFA VARIETIES AND THE POSSIBILITY OF UTILIZING THIS AS A PRACTICAL MEANS OF IDENTIFICATION. Jour. Agr. Research 21: 599–608, illus 1921.

TABLE III.—*Average length of first internode, average stem length, and average internode length of seedlings, 63 days from sowing, January 12, 1922*

[Measurements in millimeters. Lettering same as for Table II]

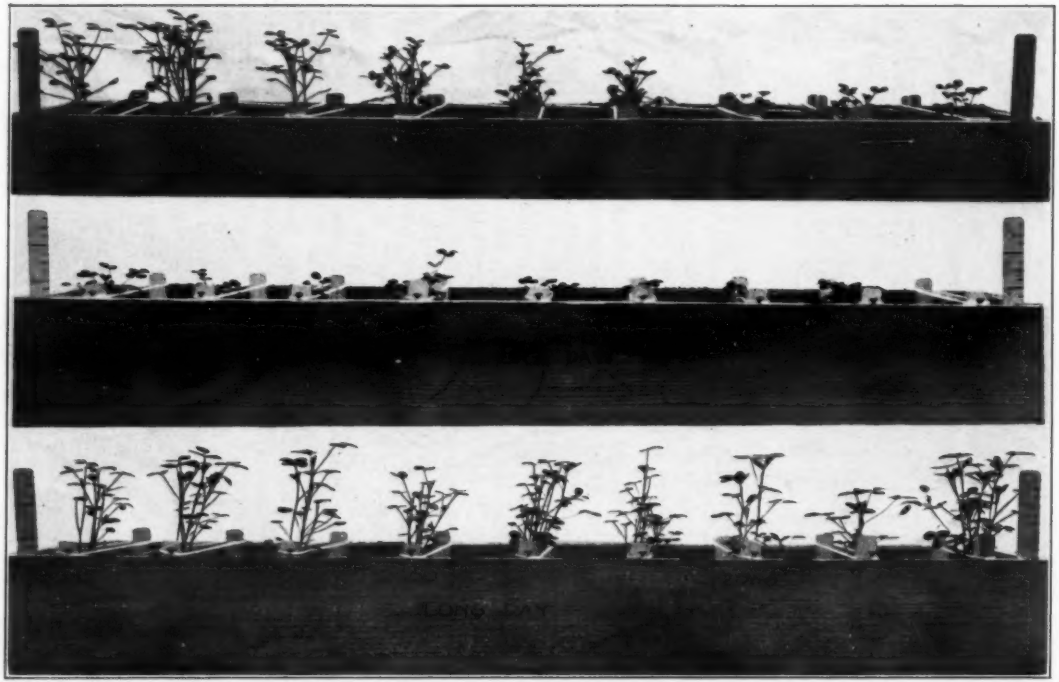
| | | 5-leaved | | | | | | 6-leaved | | | | | | 7-leaved | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Biennial | | | Annua | | | Biennial | | | Annual | | | Biennial | | | Annual | | |
| | | 2066 | 2140 | 03464 | 2143 | 12055 | 12060 | 2066 | 2140 | 03464 | 2143 | 12055 | 12060 | 2066 | 2140 | 03464 | 2143 | 12055 | 12060 |
| N | V | None. | 5.9 | 4.5 | 7 | 11.1 | 9.2 | 4.3 | 5.8 | 6 | 8.4 | 11.6 | 9.9 | 4 | 6.4 | 5.5 | None. | 11 | 9.8 |
| | S | None. | 25 | 18.2 | 26 | 45 | 45.8 | 16.5 | 30 | 23.1 | 43.8 | 55.8 | 55.9 | 25 | 49.6 | 24.7 | None. | 72.5 | 72.4 |
| | I | None. | 3.8 | 2.7 | 3.8 | 4.8 | 7.3 | 2 | 4 | 2.8 | 5.2 | 7.4 | 7.7 | 3 | 6.1 | 2.7 | None. | 8.8 | 8.9 |
| L | V | 2.7 | 3.1 | 4.6 | 5 | None. | 5.3 | 2.2 | 3.1 | 5.5 | 5 | 4.2 | 5.2 | 2 | 3.1 | 4.6 | 4.2 | 4 | 5.4 |
| | S | 9.1 | 11.5 | 15.7 | 21.4 | None. | 35.8 | 9 | 13.3 | 20.5 | 20.5 | 18 | 42.7 | 9.7 | 13.5 | 19 | 22 | 18.4 | 52.5 |
| | I | 1.3 | 1.7 | 2.2 | 3.3 | None. | 6.1 | 1.1 | 1.7 | 2.5 | 2.6 | 2.3 | 6.2 | 1.1 | 1.5 | 2 | 2.5 | 2.1 | 6.7 |
| L | V | 14.7 | 14.2 | 13.7 | 16.5 | 19 | 16 | 13.7 | 16 | 17.7 | 16 | 19.7 | 17.8 | 15 | 17.1 | 17.7 | 18 | 21.3 | 17.5 |
| | S | 73.4 | 72.6 | 73 | 76 | 93 | 89.5 | 97.3 | 106.6 | 120.5 | 96.4 | 121.2 | 117.8 | 120.7 | 142.3 | 120.5 | 135 | 157.6 | 146 |
| | I | 11.7 | 11.7 | 11.9 | 11.9 | 15 | 14.7 | 13.9 | 15.1 | 17.1 | 13.4 | 16.9 | 17 | 15.1 | 17.9 | 14.7 | 17 | 19.5 | 18.4 |

As shown in Table II, the internode length of the annual forms was, under the normal length of day, markedly greater than that of the biennial forms (pl. 2, top row), though the difference was ont as great as it had been in seedlings grown out of doors in August–October.

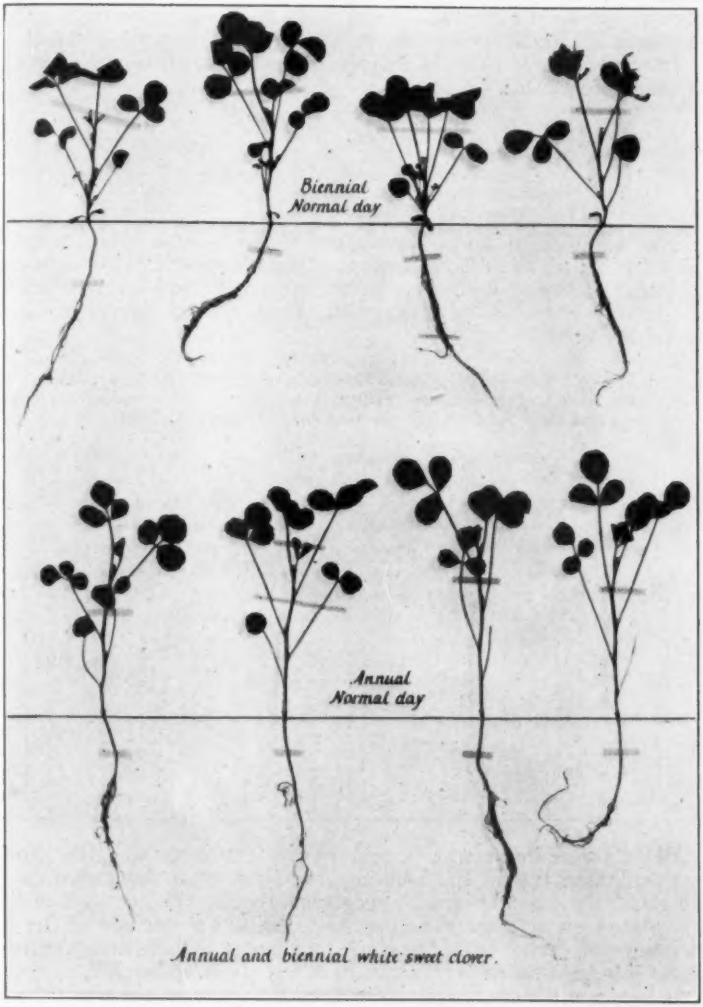
This fact is also brought out in Plate 3, which should be compared with Plate 1. The effect of decreased illumination was to shorten the internodes, both first and subsequent ones, but this shortening was, in the Arctic variety, 03464, confined to the first internode and was not shown by the subsequent internodes. Increased illumination was marked by an elongation of all internodes. For the plants recorded in Table II, and leaving out the Arctic as peculiar, the ratio of average length of internodes between annual and biennial is: For three-leaved seedlings, normal day 1 to 0.58, short day 1 to 0.54, and long day 1 to 0.86; for four-leaved, normal day 1 to 0.57, short day 1 to 0.8, and long day 1 to 0.87.

Under the long day the difference between the internode lengths of annual and biennial has been decreased, the biennials responding relatively more to the long day than the annuals. The relative size of the first internode of annual and biennial has, however, remained fairly constant under long and short days, showing that the effect of illumination has been felt mostly in the internodes after the formation of the first leaf. For the first internode the ratios of annual to biennial are as follows (Table II): Three-leaved, normal day 1 to 0.61, short day 1 to 0.69, long day 1 to 0.73; for four-leaved, normal day 1 to 0.76, short day 1 to 0.78, long day 1 to 0.73.

On January 12, 1922, a second set of the same numbers was sown and allowed to grow until March 16, 63 days after seeding. These seedlings were therefore older than those in the first set, and those under normal day had also had more hours of sunlight than those in the first set. In a few cases the number of seedlings of the seven and eight leaved sizes fell below 10 for each measurement, so these figures have less value than those in which more seedlings were available. The record for the eight-leaved seedlings is so imperfect that it is omitted (Table III).



Seedlings of biennial (2066, three rows at the right) and of annual white sweet clover grown in greenhouse December 14, 1921, to February 13, 1922, under normal day (top row), short day (middle roow), and long day (bottom row)




Annual and biennial white sweet clover grown under normal day in greenhouse January 12 to March 13, 1922. (Compare with Plate 1)

The measurements given in Table III bring out the same facts as those recorded in Table II. Under normal day the internodes of the annual were generally longer than those of the biennial (pl. 3), but this difference tended to disappear under longer illumination although, even in the older seedlings, the internodes of the annuals always averaged somewhat longer than those of the biennials. This is true of the first as well as the subsequent internodes (pls. 4 and 5).

In the winter of 1922–23 this work was repeated, the following lots of seed being used:

2062. Common biennial.
2066. Grundy County biennial.
2143. Mixed annual.
12044. Late annual.
12001. Early annual.
12056. Late annual.

The seeds were sown November 16, and some were measured January 10, 55 days from sowing; others January 25, 70 days from sowing. Those of the first set were nearly all two-leaved, those of the second nearly all three-leaved. Both sets of figures are given in Table IV:

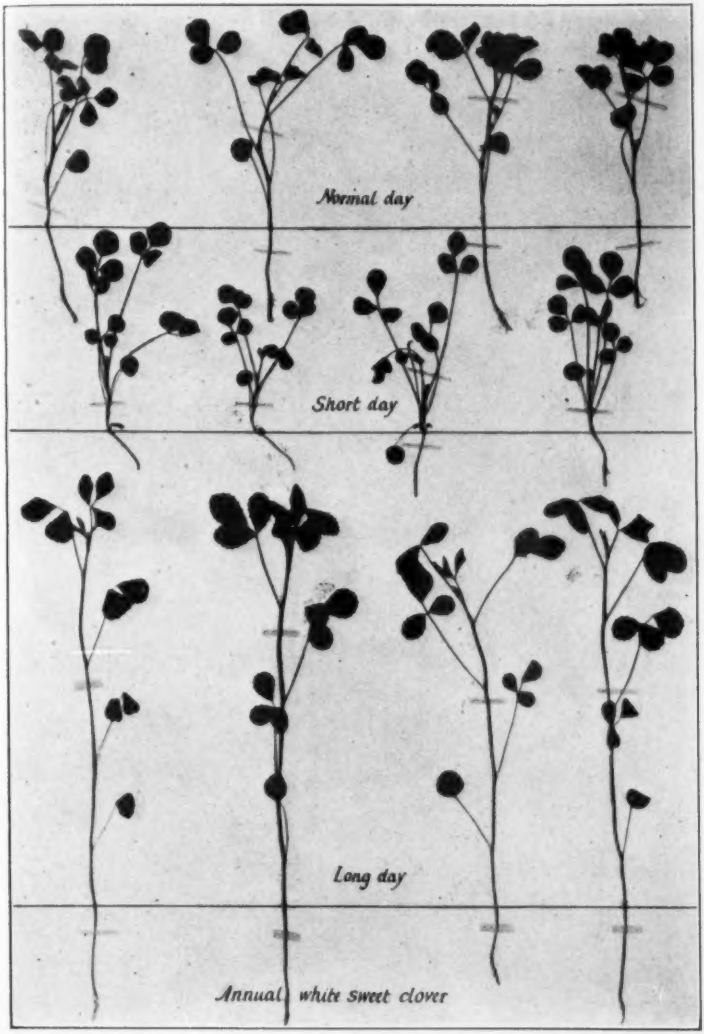
TABLE IV.—*Average length of first internode, average stem length, and average internode length of two-leaved seedlings measured 55 days and of three-leaved seedlings measured 70 days after sowing on November 16, 1922*

[Measurements in millimeters. Lettering as for Table II]

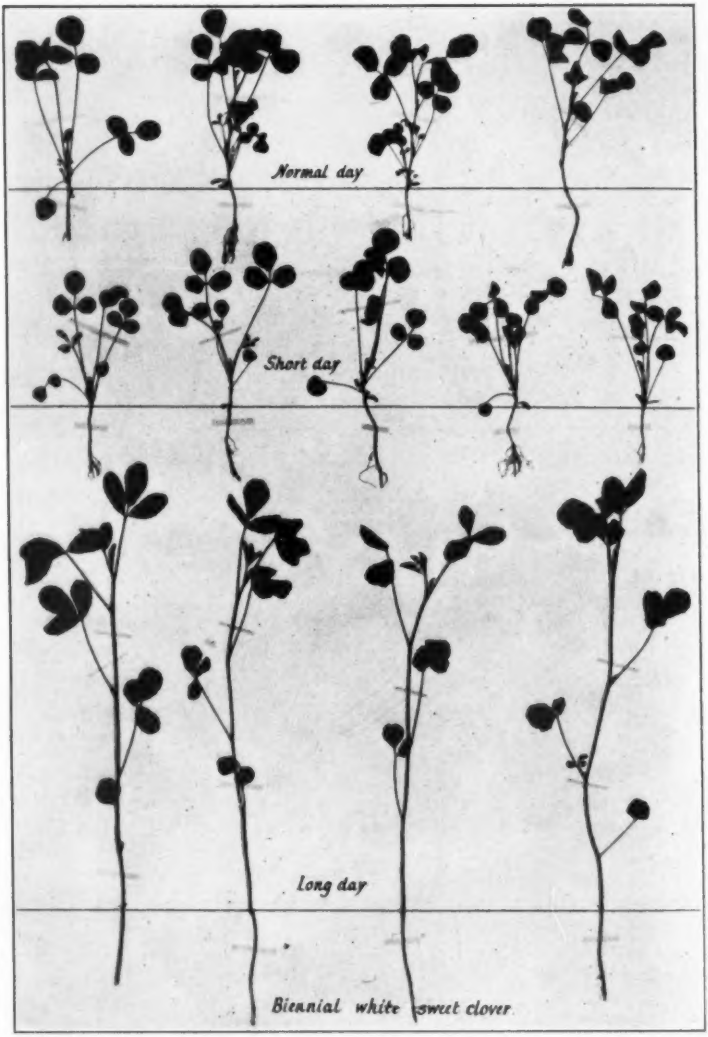
| | | 2-leaved (55 days) | | | | | | 3-leaved (70 days) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Biennial | | Annual | | | | Biennial | | Annual | | | |
| | | 2062 | 2066 | 2143 | 12044 | 12001 | 12056 | 2062 | 2066 | 2143 | 12044 | 12001 | 12056 |
| NF | I′ | 6.2 | 4.7 | 6.6 | 9 | 5.7 | 4.3 | 5 | 2.3 | 5.8 | 7 | 5.7 | 5.3 |
| | S | 10.7 | 6.4 | 11.5 | 14.7 | 12.1 | 8 | 10.6 | 6 | 17.4 | 17 | 24.5 | 13.7 |
| | I | 2.2 | .8 | 2.5 | 2.8 | 3.2 | 1.8 | 1.5 | 1.2 | 3.9 | 3.3 | 6.3 | 2.8 |
| N | I′ | 6.6 | 4.3 | 8.6 | 9.4 | 7.1 | 6.8 | 8.4 | 5 | 7.4 | 9.4 | 8 | 6 |
| | S | 15 | 6.9 | 18.2 | 18.4 | 17.3 | 14 | 21.8 | 10.2 | 24 | 28.4 | 29 | 22.2 |
| | I | 4.2 | 1.3 | 4.8 | 4.5 | 5.1 | 3.6 | 4.5 | 1.7 | 5.5 | 6.3 | 7 | 5.4 |
| S | I′ | 6.4 | 4.3 | 8 | 11.7 | 6.5 | 6.4 | 8 | 3 | 6.4 | 8 | 7 | 6.1 |
| | S | 10.6 | 6 | 15 | 20 | 14.2 | 12.4 | 17 | 4.7 | 13.4 | 17.4 | 23 | 15 |
| | I | 2.1 | .8 | 2.5 | 8.6 | 3.8 | 3 | 3 | .6 | 2.3 | 3.1 | 5.3 | 3 |
| L | I′ | 16.5 | 12.3 | 15.5 | 22.4 | 13.4 | 12.1 | 15 | 11.6 | 15 | 23 | 14 | 13 |
| | S | 35 | 28 | 35 | 46.8 | 29.9 | 26 | 37.6 | 39 | 49.3 | 65 | 48 | 45 |
| | I | 9.2 | 7.8 | 9.8 | 12.2 | 8.3 | 6.9 | 7.5 | 9.1 | 11.4 | 14 | 11.3 | 10.7 |

All flats were in a center bench, some 6 feet below the glass, and it was suggested that if the seedlings, under normal day, were nearer the glass, the stems might be shorter. To test this, one set of flats was placed on a frame raised so as to bring the surface of the soil within 8 or 9 inches from the glass. In Table IV, the measurements of the seedlings taken from these flats are given under NF.

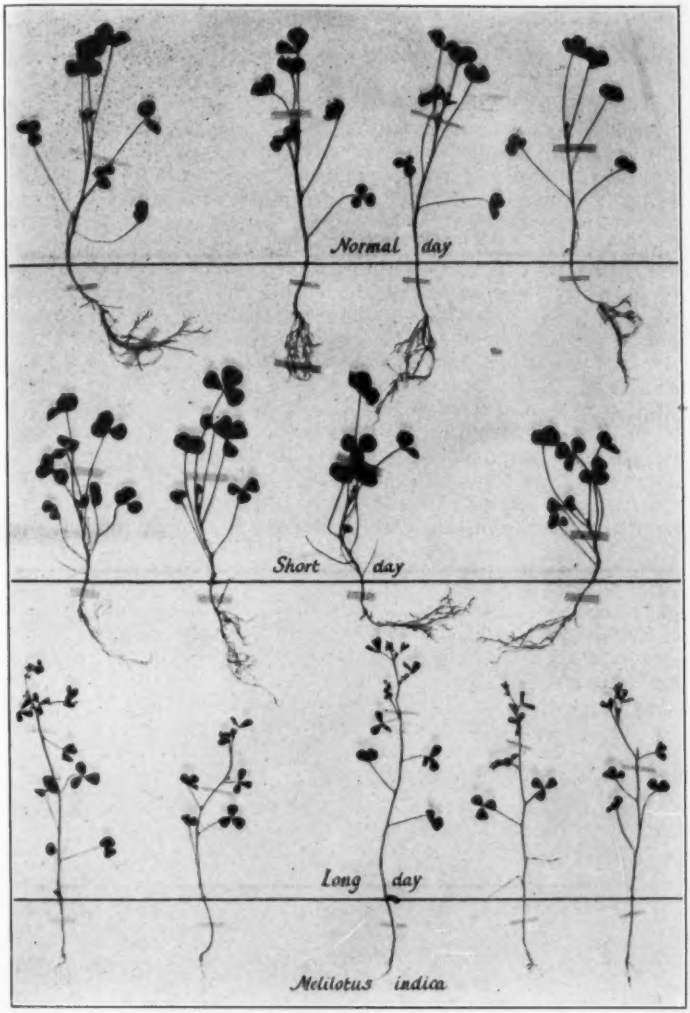
As between the seedlings, under normal day, from the center bench and those from the raised bench, the length of the first internode does not show any consistent difference in the younger seedlings, but does in the older ones. In the three-leaved seedlings the first internode of those from the raised bench is regularly and noticeably shorter than in those seedlings growing farther from the light. This feature comes out more prominently when the stem lengths of the two sets




Annual white sweet clover seedlings grown under normal, short, and long day in greenhouse January 12 to March 13, 1922, showing the effect of photoperiodicity




Biennial white sweet clover seedlings grown under normal, short, and long day in greenhouse January 12 to March 13, 1922, showing the effect of photoperiodicity




Seedlings of *Melilotus indica* grown under normal, short, and long day, November 16, 1922, to February 17, 1923, showing effect of photoperiodicity. Note the small leaves, blossoms, and young pods on plants 93 days from seeding

are considered. In both two-leaved and three-leaved seedlings the plants from the raised bench are shorter than those grown farther from the light. In many cases this length is nearly the same as that secured under the short day.

Comparing these two sets with those grown under normal day at about 6 feet from the glass, it will be noted that the effect of growing the seedlings near the glass has been expressed in the same direction and often to the same degree as has that of decreasing the time of illumination. Increasing the duration of illumination has again resulted in a marked increase in length.

One trial with *Melilotus indica* may be mentioned here as an example of the striking effect photoperiodicity may exert. Seeds of this species were sown November 16, 1922, and the seedlings removed and pressed February 17, 1923. The behavior of those under short day was not at all remarkable—indeed they can not be readily distinguished from those grown under normal day—but the seedlings under long day show striking changes (pl. 6). Not only have the internodes been lengthened, but the plants have flowered and fruited when only 93 days old and barely 9 to 11 cm. long. Moreover, the leaflets are decidedly smaller than those of seedlings grown under normal day. This behavior of *Melilotus indica* under long day is quite in harmony with its field behavior. When grown in the South, it blooms as soon as the days get longer in spring, and in the North it has no value because it runs to bloom when only a few inches high. It has been thought that the warm days of a northern May accounted for this, but it now seems probable that the long period fo sunshine rather than the temperature is the exciting cause.

## CONCLUSION

It has been shown that the internodes of the annual white sweet clover are longer than those of the biennial. This is most marked in seedlings grown out of doors in late summer and is then sufficient in degree to serve to distinguish the two. Under a short or a long day in the greenhouse the differences tend to disappear, and controlling the period of illumination can not be recommended as a useful method in this case, though Oakley and Westover found it useful in alfalfa varieties.